EFFECTS OF THERMAL OXIDATION ON THE CONSTITUTION OF BUTTERFAT, BUTTERFAT FRACTIONS AND CERTAIN VEGETABLE OILS

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ABSTRACT

EFFECTS OF THERMAL OXIDATION ON THE CONSTITUTION OF BUTTERFAT, BUTTERFAT FRACTIONS AND CERTAIN VEGETABLE OILS

Donna B. Kupranycz McGill University, 1986

The present study was undertaken to characterize the nonvolatile degradation products which are formed during the thermal oxidation of butterfat and butterfat fractions as well as certain vegetable oils (soybean, sunflowerseed, canola and corn oils). Samples of winter and summer butterfat, liquid and solid butterfat fractions (obtained by a laboratory method which was developed and is described), and the vegetable oils were heated at 185°C in the presence of air for 8 and 16 h.

The results of various analyses performed on the nonvolatile fractions of the heated fats and oils indicated that butterfat is much more stable to thermal oxidation than canola, sunflowerseed and soybean oils. This was evidenced by substantially higher contents of inter- and intramolecular polymeric fatty acids and total polar components in the vegetable oils than in any of the butterfat samples after both 8 and 16 h of heat treatment. The corn oil also exhibited a high degree of stability to thermal oxidation after 8 h of heating. The 16-h corn oil data, however, was less certain due to the presence of a very viscous and dark coloured material on the inner walls of the oxidation flask which was not

soluble in chloroform, ethyl ether, or acetone; this was believed to contain highly polymerized oil and was not observed with any of the other samples.

All of the vegetable oils, after both 8 and 16 hours of heat treatment, contained substantially higher levels of C18 cyclic fatty acids than did any of the butterfat samples which were treated under identical conditions.

With the butterfat fractions, both the solid and liquid fractions exhibited a greater stability to thermal oxidation compared to whole butterfat. The results of this study suggested that the high degree of stability of butterfat to thermal oxidation is due to its fatty acid composition (i.e., the high level of saturated fatty acids in butterfat) and also to an antioxidant component (or components) which is (are) concentrated in the liquid fractions during butterfat fractionation.

The results also indicated that the principal routes of decomposition are different in liquid and solid butterfat fractions. The liquid fractions contained higher levels of dimeric and higher oligomeric triglycerides in their degradation products while the solid fractions contained higher levels of oxygenated triglycerides and/or hydrolysis products.

RESUME

LES EFFETS THERMO-OXYDATIFS SUR LA COMPOSITION CHIMIQUE DE LA MATIÈRE GRASSE DU BEURRE ET DE SES FRACTIONS, AINSI QUE SUR CERTAINES HUILES VÉGÉTALES.

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Nous avons entrepris la présente étude dans le but de caractériser les composés non volatiles produits lors du traitement thermo-oxydatif de la matière grasse du beurre et de ses fractions ainsi que de certaines huiles végétales (soya, tournesol, colza et maïs).

Des échantillons de matière grasse prélevés à partir du beurre d'hiver et du beurre d'été, des fractions liquides et solides de ce gras (recueillies à l'aide d'une technique developeé dans nos laboratoires et décrite dans ce travail), et différentes huiles végétales ont été chauffés à 185°C et combinés à l'action d'oxygène durant deux périodes de 8 et 16 heures.

Les résultats de l'analyse des composés non volatiles produits suite aux modifications thermo-oxydatives de ces corps gras indiquent une plus grande stabilité de la matière grasse du beurre aux altérations thermiques et oxydatives comparativement aux huiles de soya, de colza et de tournesol soumises au même traitement.

L'examen de la composition chimique de nos échantillons accentue d'avantage ces différences. Ainsi, la teneure en polymères (intra et inter moléculaire) d'acides gras et en composés polaires des huiles végétales ayant subit des modifications thermo-oxydatives est supérieure à tous les autres échantillons de matière grasse du beurre, et ça, peu importe la durée du traitement. L'huile de maïs démontre également une grande stabilité aux altérations oxydatives après 8 heures de traitement. Par contre, après les 16 heures de traitement de cette huile (maïs), la présence d'une couche liquide visqueuse et foncée de matériel rend difficile l'interprétation des résultats puisqu'il nous a été impossible de retirer cette substance du ballon oxydatif; nous croyons que cette substance, absente des autres échantillons, renferme une huile hautement polymérisée.

Après le traitement thermo-oxydatif, toutes les huiles végétales contiennent une plus grande quantité de monomères cycliques que la matière grasse du beurre ayant subit les mêmes conditions.

Les fractions à la fois solides et liquides de matière grasse du beurre sont d'avantage stables après traitement thermo-oxydatif que la matière grasse d'origine. Ces résultats démontrent que la composition en acides gras de la matière grasse du beurre determine son susceptibilité à la dégradation thermo-oxydative, (c'est-à-dire, une teneure élevée en acides gras saturés), ainsi que le ou les antioxydants qui se retrouvent en majorité dans la fraction liquide du beurre lors du fractionnement de la matière grasse d'origine.

Les résultats indiquaient que les principales routes de dégradation sont différentes dans les fractions liquides et solides de la matière grasse du beurre. Les produits formés dans la fraction liquide se composent d'avantage de dimères et d'oligomères de triglycerides, alors que l'on retrouve une teneure plus élevée de triglycérides oxydés et/ou de produits hydrolysés dans la fraction solide de matière grasse du beurre.

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CHAPTER I

GENERAL INTRODUCTION

Thermal oxidation of a fat (ca. 180°C - 200°C in the presence of air), as when fats are used in frying, is known to result in a variety of complex chemical reactions in the fat of the oxidative and thermolytic types (Nawar, 1985). This leads to the formation of numerous volatile and nonvolatile degradation products, many of which are important from the standpoints of flavour, odour and nutrition.

Of particular interest in terms of the nutritional value of heated fats, are the nonvolatile degradation products which accumulate in the thermally oxidized fats and are subsequently ingested with the food. Studies have shown that more of the nonvolatile degradation products are found in the food than in the remaining frying oils on a proportionality basis (Hussain and Morton, 1974; Thompson and Aust, 1983). A relationship between the nonvolatile, non-urea-adduct-forming fraction of thermally oxidized vegetable oils and various toxic responses has been suggested by the animal experiments conducted by Crampton et al. (1953, 1956). The work of subsequent researchers (Michael et al., 1966; Artman, 1969; Artman and Smith, 1972; Ohfugi and Kaneda, 1973) supports these findings and suggests that the nonadductable monomers (cyclic monomers) and oxidative dimers are the main source of toxicity. The major question which continues to be disputed is the extent to which these newly formed compounds are formed during normal deep frying or other procedures involved in food preparation and the significance of these compounds in terms of human health.

There is some evidence in the early literature that butterfat may have nutritional advantages over vegetable oils as a cooking fat (Johnson et al., 1956; Bhalerao et al., 1959; Coombs et al., 1965). There is, however, very little information on the composition of the nonvolatiles in heated butterfat; this type of information may help to explain the observed nutritional advantages.

In this investigation, the thermal oxidative behaviour of butterfat was studied and compared to those of canola, soybean, sunflowerseed and corn oils. In addition, butterfat from both winter and summer butter, was fractionated by crystallization from molten fat and the thermal oxidative behaviour of the resultant fractions (solid and liquid) was studied. Fractionation of butterfat is of particular interest to the Canadian Dairy Industry as it may lead to an expansion in the use of butterfat in food formulation and processing. To the authors' knowledge, there is no information in the literature on the thermal oxidative behaviour of such butterfat fractions. To obtain meaningful data from the study of butterfat fractions and since butterfat fractions are available only to a limited extent in some European countries, a laboratory procedure which closely resembles that used in the Tirtiaux industrial process (S.A. Fractionnement Tirtiaux, Belgium), was developed. The first part of this thesis deals with the description of this process and characterization of the fractions which were produced. The next and subsequent sections deal with the thermal oxidation of the various butterfat and vegetable oil samples and the characterization of the resultant nonvolatile fractions (dimeric and higher oligomeric material, polar and nonpolar material, and C18 cyclic monomers) according to their composition.

CHAPTER II

LITERATURE REVIEW

1. COMPOSITION OF BUTTERFAT

Among all of the naturally occurring fats, butterfat from cow's milk is the most complex in its chemical and physical characteristics. Adding to this complexity is the variability in its composition resulting from feeding conditions, stage of lactation and breed of the animal. A detailed discussion of the chemistry of milkfat is not within the scope of this thesis, but since fractionation and thermal oxidation of butterfat are greatly affected by composition, some general remarks on the composition of the lipids in milk are included here.

A typical composition of the lipids in whole bovine milk is given in Table 1.

Table 1. Composition of Lipids in Whole Bovine Milk (Jensen, 1973).

Lipid	Weight %
Hydrocarbons	trace
Sterol esters	trace
Triacylglycerols	97 - 98
Diacylglycerols	0.28 - 0.59
Monacylglycerols	0.016 - 0.038
Free fatty acids	0.10 - 0.44
Free sterols	0.22 - 0.41
Phosphoplipids	0.20 - 1.0

Milk lipids also contain appreciable quantities of the lipid soluble vitamins, i.e., mainly vitamins A, D, E and K. The amounts of these vitamins in cow's milk as a percentage of the total lipids have been reported as follows: 0.0006 - 0.0009% Vit A; 0.00000085 - 0.0000021% Vit D; 0.0024% Vit E; 0.0001% Vit K (Kurtz, 1974).

The lipids in milk occur in the form of globules (ca. 2 - 3 μ in diameter) which are surrounded by a specialized bilayer membrane composed primarily of proteins, phospholipids, glycolipids, sterols and acylglycerols (Patton and Keenan, 1975). This membrane, known as the milkfat globule membrane (MFGM), maintains the integrity of the globules and renders them compatible with their aqueous environment. The fat globule core consists almost entirely of triacylglycerols (98 - 99%); more than 95% of the total milk lipid is in the globule fraction and the remainder is present in the MFGM (Table 2).

Table 2. Composition of Lipids from Milkfat Globule Membrane (Bracco et al., 1972).

Lipid component	% of membrane lipids
Carotenoids	0.45
Squalene	0.61
Cholesterol esters	0.79
Triglycerides	53.40
Free fatty acids	6.30[a]
Cholesterol	5.20
Diglycerides	8.10
Monoglycerides	4.70
Phospholipids	20.40

[a] Contained some triglycerides

When milk or cream is churned to make butter, the MFGM's are disrupted and the more water soluble components (proteins) plus approximately half of the phospholipids are lost in the buttermilk (Lampert, 1975). Hence the composition of butterfat differs from the parent milkfat, particularly in terms of the content of phospholipids.

Butterfat contains a greater number of different fatty acids than any other food fat; at least 437 have been listed in a comprehensive review paper by Patton and Jensen (1975). The following fatty acids are believed to be present in butterfat: even- and odd-numbered saturates from C2 to C28; even- and odd-numbered monoenes from C10 to C26 (except C11), including positional and geometric isomers; even-numbered polyenoic acids (C18:3, C18:4, C20:3, C20:4, C20:5, C22:3, C22:4, C22:5, C22:6), including some conjugated trans isomers; iso and anteiso monomethyl-branched fatty acids from C16 to C28 with three to five methyl branches; keto- and hydroxy-fatty acids (including many positional isomers, and saturated and unsaturated acyl chains); and at least one cyclic acid. Of this large number of fatty acids, approximately 20 are major fatty acids; the remainder are minor and occur in small or trace quantities (Table 3).

Table 3. Fatty Acid Composition of Milkfat (Walstra and Jenness, 1984).

Acid	Notation	Composition (in mole %) of neutral glycerides
Saturated		69.0
Butyric	4:0	8.5
Caproic	6:0	4.0
Caprylic	8:0	1.8
Capric	10:0	3.0
Lauric	12:0	3.6
Myristic	14:0	10.5
Palmitic	16:0	23.5
Stearic	18:0	10.0
Odd-numbered		2.5
Branched		1.1
Other		0.7
Monoene		27.0
Palmitoleic	16:1 Δ 9c	1.4
Oleic	18:1 Δ 9c	21.0
Other		5.5
Diene		2.5
Linoleic	18:2 Δ 9, 12c	1.8
Other		0.7
Polyene		0.8
α-Linolenic	18:3 Δ 9, 12, 15c	0.4
Other		0.4
Keto		0.3
Hydroxy		0.3
Fatty alcohol		0.01
Fatty aldehyde		0.02

2. THE CRYSTALLIZATION BEHAVIOUR OF BUTTERFAT AND SOME ASPECTS OF BUTTERFAT FRACTIONATION

The melting and solidification behaviours of butterfat are as complicated and variable as the chemical composition of the fat. Because of the broad spectrum of fatty acids in butterfat, a large number of different triacylglycerols can be formed; consequently the melting and crystallization occurs over a wide range of temperature. It is normally anticipated that butterfat is liquid above 40°C and completely solidified below -40°C (Brunner, 1974). At intermediate temperatures it is a mixture of solid and liquid fats.

Milkfat has been used traditionally, for the most part, as butter, which is an important food-fat product in the dairy industry. The wide melting range of butterfat, however, limits its usefulness in non-dairy food applications which require specific or sharp melting characteristics; this puts butterfat at a disadvantage when compared to the wide range of specialty vegetable fats and oils which are available to the food-fat industry. One way to overcome this limitation is to modify the chemical and physical characteristics of butterfat. Methods which have been attempted in this direction include (i) fractionation of butterfat into high-melting and low-melting fractions (de Man, 1961), (ii) blending of butterfat with vegetable oil (for example, in the manufacture of dairy blends to improve low temperature spreadability) (Amer and Myhr, 1973), and (iii) the incorporation of polyunsaturated vegetable oils into the

diet of the cow to increase the degree of unsaturation of the milkfat (Scott et al., 1970). Only the first method, fractionation, is discussed here.

Fractionation is a process involving crystallization and separation; the component triglycerides of fats and oils are separated, usually as mixtures, by partial crystallization in a liquid phase followed by separation of the crystalline fat from the liquid oil (Thomas, 1985). It is usually considered that the three following successive stages are involved: (i) cooling of molten fat to produce nucleation; (ii) growth of crystals to a size and shape that permit efficient separation; and (iii) separation or isolation and purification of the resultant solid and liquid phases (Thomas, 1985).

Fractionation of butterfat can be made either by crystallization of the fat dissolved in an organic solvent such as acetone or alcohol (Chen and de Man, 1966; Colombini et al., 1979; Larsen and Samuelsson, 1979) or by direct cooling of the melted fat (de Man, 1968; Black, 1973 and 1975; Schaap and Rutten, 1976; de Man and Finoro, 1980; Badings et al., 1983 a and b). Although recrystallization from solvents yields a more distinct separation of the crystalline and liquid fractions, the latter method is preferred for food industry applications because of (a) flavour and toxicological problems that may result from solvent residues; (b) the high cost of solvent recovery; and (c) the possibility of destruction of desirable aroma components and vitamins.

An understanding of the nature of triglyceride crystallization is important for optimal butterfat fractionation. According to Mulder and Walstra (1974), bulk milkfat contains sufficient catalytic impurities

(e.g., monoglycerides) to initiate heterogeneous nucleation with little supercooling. Tverdokhleb et al. (1974) stated that a liquid-crystal phase is formed in the melt during cooling after which a monocrystalline nucleus emerges from the high-melting glycerides. The nucleus has the shape of a spherolite consisting of crystals radiating outward from a common center. During the growth of the spherolite, the needle-shaped crystals which radiate outwards, thicken and take on a feather-like structure characteristic of a typical spherolite.

A number of factors influence the growth of crystals in the liquid fat and consequently the shape and size of crystals. According to Tverdokhleb et al. (1974), the formation of spherolites in bulk milkfat appears to be a rhythmical process due to the thermal energy (i.e., heat of crystallization) which is emitted as crystallization proceeds. As heat is emitted, the concentration of glycerides which are crystallizable under the given conditions, decreases. The growth of the spherolite will therefore stop temporarily until a state of equilibrium is reached between glycerides which are crystallizing and crystals which are redissolving, and the heat emitted is dissipated. This results in crystals which are arranged along the outer surface and this gives the spherolite the shape of concentric waves. The shape of the crystals which are formed may be hexagonal (α form), orthorhombic (β' form) or trielinic (β form); this will depend on whether they crystallize in the unstable or more stable polymorphic form. On rapid cooling of fats, α crystals (the least stable form) usually form first (Woodrow and de Man, 1968) because of their relatively simple structure. The β and β' crystals form during

further crystallization, or directly when cooling is slower. Polymorphic transitions are possible; this implies alteration of the crystal lattice structure (solid ⟶ solid transition) (van Beresteyn, 1972) with the liquid form as an essential intermediary (Mulder and Walstra, 1974). Thus polymorphic transitions are more probable when temperature fluctuations occur (e.g., when heat is emitted during crystallization). The size of the butterfat crystals depends largely on the conditions which are employed during crystallization, in particular the rate of cooling and degree of agitation. If molten fat is cooled rapidly, many small crystals with a maximum diameter of 1 - 2 μ are formed while slow cooling results in the formation of a fewer number but larger crystals with diameters up to 40 μ (de Man, 1961). Very low and very high agitation speeds result in the formation of very fine crystals (Schaap and Rutten, 1976). In the absence of stirring, the fat crystals which are suspended in liquid oil tend to flocculate into a network held together by van der Waal's forces (van den Tempel, 1961). Slow stirring speeds (10 - 50 rpm) have been shown by several workers (de Man, 1968; Black, 1975; Schaap and Rutten, 1976) to result in the formation of large crystals; this is the most desirable size for optimal separation of solid and liquid fractions. When crystallization is so far advanced that almost all of the remaining liquid phase is bound into the network, the mass appears as a complete solid and the liquid phase cannot be separated (Mulder and Walstra, 1974).

The composition and physical properties of the fat fractions which are obtained depend on the cooling procedure as well as on the efficiency of separation. Even under optimal conditions of separation, the crystallization of butterfat directly from liquid fat results in part of the liquid

fat enclosed within the spherolites; this gives rise to a large volume/mass ratio with the consequence that a relatively large amount of liquid fat is adsorbed to the surfaces of the fat crystals (Larsen and Samuelsson, 1979).

In recent years, butterfat has been fractionated on an experimental level by an extraction process which is based on the use of a liquified gas (CO_2) or a gas in the supercritical state (Timmen et al., 1984; Biernoth and Merk, 1985). Fractionation by supercritical CO_2 differs from fractionation by crystallization from molten fat in that the triglycerides are separated according to their molecular weight and particularly, by their carbon number, rather than according to the degree of saturation of the fatty acid residues in the triglycerides. The production of butterfat fractions which contain primarily short chain fatty acids (C4 to C10) is of interest for dietary purposes (Timmen et al., 1984).

3. SOME GENERAL ASPECTS OF THERMAL OXIDATION

Thermal oxidation of a fat results in the formation of numerous degradation products, both volatile and nonvolatile. Numerous investigations have dealt with the thermal oxidative behaviour of vegetable fats and oils; these oils and fats are used in considerable quantities for deep fat frying of foods and thus a knowledge of their decomposition is of great practical and nutritional importance.

The reaction pathways which occur during thermal oxidation, the rates of these reactions, and the products which are formed depend on the

interaction of many factors such as degree of unsaturation of the fat, time and temperature of heating, frequency of heat treatment (i.e., intermittent or continuous heating), surface to volume ratio, and the presence of food, moisture, prooxidants or antioxidants in the fat (Perkins, 1976; Alexander, 1981; Gere, 1982). It has also been suggested that the positional distribution of fatty acids in the triglycerides may have an influence on the relative rates of oxidation of fats (Lau et al., 1982; Wada and Koizumi, 1983).

The principal reaction pathways which occur under thermal oxidative conditions are believed to be the same as those which occur under autoxidative conditions, i.e., via the formation and decomposition of hydroperoxide intermediates (Nawar, 1985). At elevated temperatures, however, hydroperoxide decomposition is extremely rapid, resulting in relatively complex decomposition patterns with less predictability of products as compared to when autoxidative reactions occur (Nawar, 1985). The basic mechanisms for hydroperoxide formation from unsaturated fatty acids under autoxidative conditions, have been reviewed (Frankel, 1980; Frankel, 1984).

Saturated fatty acids are considerably more stable toward oxidation than are unsaturated fatty acids. However, when the temperature is raised beyond 150°C, saturated fatty acids are subject to oxidative as well as thermal degradation (Brodnitz et al., 1968a). The thermal oxidation of saturated fatty acids is dealt with in more detail in another section.

With unsaturated fatty acids, the predominant thermolytic reaction products are dimers, higher oligomers and cyclic compounds. When heating

occurs in the presence of air, oxidative reactions become superimposed resulting in polarity differences in these materials due to the presence of hydroxyl, carbonyl, epoxide, and other oxygen-containing groups as well as ether and peroxide bridges (Ottaviani et al., 1979). Unlike the polar dimers and cyclic monomers which have been shown to be relatively toxic (Ohfugi and Kaneda, 1973; Iwaoka and Perkins, 1976 and 1978), polymeric material of relatively high molecular weight, is not considered to be toxic since it is not absorbed by the body (Michael et al., 1966; Nolen et al., 1967; Poling et al., 1970). The formation of polymeric material is, however, important since these materials darken the colour of the oil and increase its viscosity. In addition, since polyunsaturated fatty acids are preferentially consumed in polymerization reactions (Pokorny et al., 1976b), their content in the oil is reduced (Gere, 1982; Thompson and Aust, 1983).

4. CHARACTERIZATION OF POLYMERS FROM MODEL SYSTEMS OF FATTY ACID ESTERS AND GLYCERIDES

Separation and characterization of polymeric materials derived from heated oils is difficult. If one considers the variety of fatty acids in the oil, their different degrees of unsaturation, the variety of positional arrangements of the triglycerides, the numerous points where oxygen may attack the molecule, and the multiple routes of hydroperoxide breakdown, it is obvious that a multitude of chemical compounds may be formed. Furthermore, the dynamic nature of chemical changes that occur

during thermal oxidation means that any products which are isolated will generally reflect only the situation at the time of sampling and the study is limited to the compounds most amenable to separation. For these reasons, most of the information about the types of products which could be expected when fats are heated come from studies of pure triglycerides or fatty acid esters.

4.1 Dimers

Dimeric polymers have been identified as a major component of the nonvolatile products which are formed in thermally oxidized fats. Catalysts which are important in dimerization reactions, are those which generate free radicals such as ultraviolet light, peroxides, and metals (Evans et al., 1965). Considerably more information has been obtained about the structure of purely thermal polymers (i.e., those which are formed in the absence of oxygen), than polymers that are formed under oxidative or thermal oxidative conditions. Thermal polymerization reactions have been used commercially in the production of dimerized fatty acids such as those found in drying oils used in paints and coatings (Johnson, 1979). As early as 1929, Scheiber postulated that the isolated double bonds of polyunsaturated fatty esters, conjugate prior to their dimerization during polymerization reactions (200°C - 300°C) in the absence of oxygen (Johnson, 1979). In 1933, Kappelmeier proposed a Diels-Alder mechanism to explain the polymerization reactions that take place between conjugated esters and non-conjugated esters (Johnson, 1979).

Wheeler and White (1967) used mass spectrometry to show the presence of monocyclic, bicyclic, and tricyclic dimeric structures when

methyl linoleate was heated (290°C) in an evacuated tube for 48 h. The authors proposed that the monocyclic dimer forms as a result of isomerization of the non-conjugated ester to the conjugated ester which then dimerizes with a second linoleate molecule by a Diels-Alder mechanism. The bicyclic structure was thought to result from a hydrogen transfer free radical coupling mechanism followed by rapid intramolecular cyclization; the tricyclic dimer was formed from intramolecular alkylation of bicyclic structures.

Other researchers have suggested that the Diels-Alder mechanism is favoured when linoleic acid is the substrate but that a free radical mechanism is most likely operative when oleic acid is degraded thermally. According to the latter mechanism, a transfer of a hydrogen radical from one molecule to another forms two organic radicals that combine to form either an acyclic dimer or a six-membered ring (Johnson, 1979).

Figure 1 gives some possible structures of thermal dimers; this figure gives only one of the many isomers that are possible if chain length, double bond position and the orientation of the hydrocarbon and ester chains on the ring are ignored.

Figge (1971), using radiotracer techniques, studied the mechanism and kinetics of formation of dimeric fatty acid methyl esters from a mixture of methyl oleate and methyl cis, cis-linoleate (14:86 w/w) at 280°C (vacuum, for 72 h). The author concluded that the formation of dimeric fatty acid methyl esters under thermal conditions proceeded predominantly between like esters with the methyl oleate and methyl linoleate reacting with each other only to a limited degree. The rate of

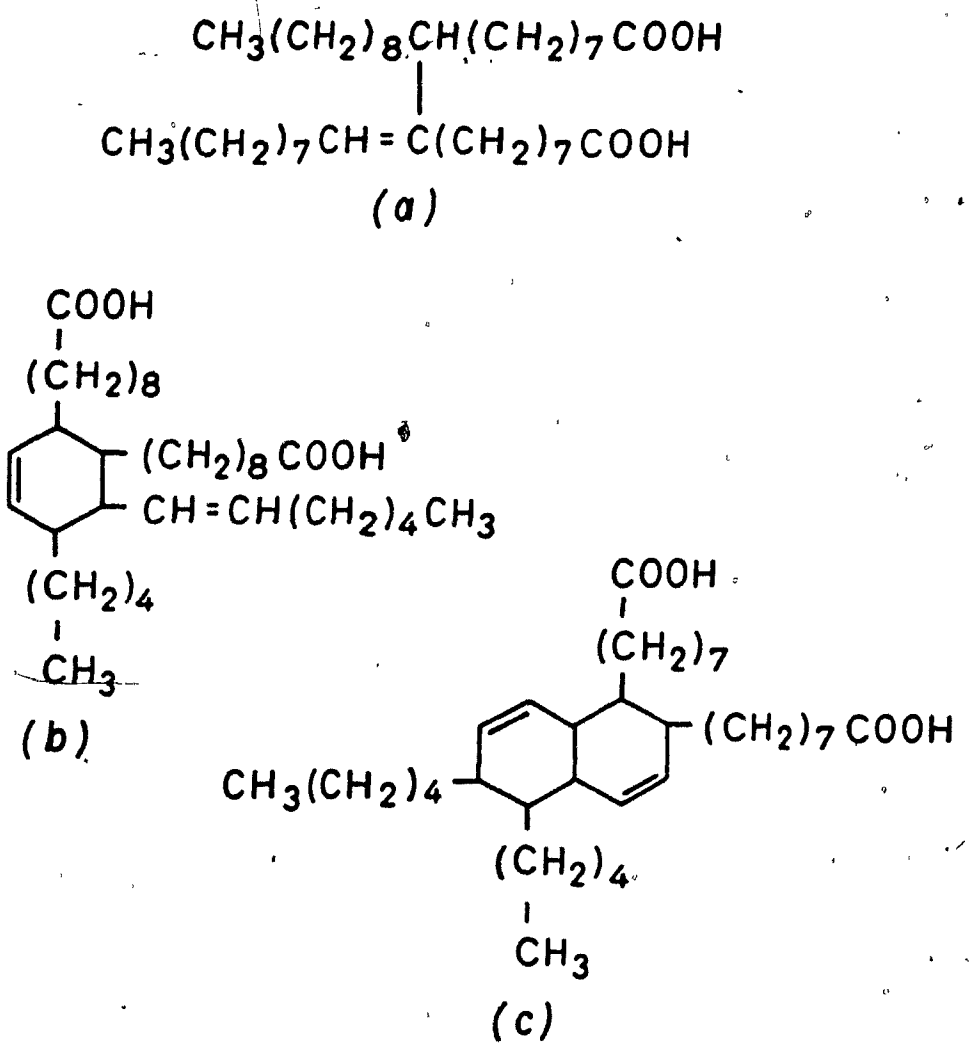


Fig. 1. Some structures of thermal dimeric acids; (a) acyclic dehydrodimer, (b) monocyclic and (c) bicyclic. (From Johnson, 1979)

formation of methyl linoleate - methyl linoleate esters was greater than that of methyl oleate - methyl oleate esters. By the use of silica gel G layers impregnated with silver nitrate (10%), Figge observed that methyl oleate and methyl cis, cis-linoleate tended towards an isomerization equilibrium with methyl elaidate and an unknown isomer of methyl linoleate respectively. Whether cis or trans isomers were consumed preferentially in the dimerization reactions could not be ascertained.

Dimers prepared by oxidation have a greater relative polarity than do thermal dimers due to the presence of hydroxyl, carbonyl, and other oxygen containing groups. These polarity differences can be used as a basis for differentiating between oxidative and thermal dimers (Evans et al., 1965). When heat is applied during oxidation, polymerization reactions become very complex, not only through free radical reactions of the decomposing hydroperoxides, but by simultaneous formation of thermal dimers and through combinations of different active monomeric materials, many of which contain oxygen (Evans et al., 1965).

Fatty acid hydroperoxides are effective catalysts for free radical polymerization or they may themselves take part in the polymerization reaction. The temperature of oxidation and the environment of peroxide breakdown appear to be important factors that determine the role of hydroperoxides in polymerization reactions and are most likely responsible for the diversity of results that are reported in the literature.

Frankel and his co-workers (1960) noted that when methyl linoleate hydroperoxides were decomposed by heating at 210°C for 15 min under nitrogen, there was a linear relationship between the concentration of

hydroperoxides and the yield of dimers. They concluded that only the hydroperoxides participate in the polymerization reaction; the dimers were linked by a C-C bond and contained 1 mole hydroxyl, 0.5 mole carbonyl, two double bonds and perhaps some epoxide or intramolecular peroxide per mole of dimer. There was no evidence to suggest a six-membered cyclic structure and they postulated that the homolytic cleavage of the hydroperoxy group to alkoxy and hydroxy radicals was responsible for the observed reactions. These results are in contrast to those of Williamson (1953); this author showed that the dimer formed during thermal decomposition of methyl linoleate hydroperoxides in the presence of excess methyl linoleate (100°C, 23 h, under nitrogen), arises from the union of two methyl linoleate molecules joined by a C-C bond. Williamson suggested that the hydroperoxide acted as an initiator of the free radical reaction. Later, Mounts et al. (1970) who used radioactive tracer techniques showed that dimers were produced from one molecule of methyl linoleate and one molecule of methyl linoleate hydroperoxide. In this study, a mixture of methyl linoleate hydroperoxide (5%) and methyl linoleate was decomposed by heating at 210°C (10 min, N_2). The extent of ester-hydroperoxide interaction was determined by considering the specific activities of the dimers formed when ^{14}C-labelled hydroperoxide was decomposed with methyl linoleate; unlabelled hydroperoxide was also decomposed with ^{14}C-labelled linoleate. This diversity of results may be due to the different temperature conditions and the amount of hydroperoxide relative to methyl ester in the reaction mixture.

Pokorny et al. (1976a) showed that free carboxyl groups of fatty acid hydroperoxides act to increase the rate of reaction and thus may

themselves participate in the polymerization reaction. These workers heated mixtures of butyl oleate hydroperoxide and palmitic acid (10:90 or 50:50 ratio) under nitrogen, at temperatures ranging from 60°C to 120°C. The decomposition of butyl oleate hydroperoxide in palmitic acid followed first order reaction kinetics which is contrary to the reaction of 10% butyl oleate hydroperoxide in butyl palmitate or butyl oleate which followed second order kinetics. The activation energy of the hydroperoxide decomposition was 70.4 kJ/mole in the medium of palmitic acid and remained constant within the temperature range that was studied. The proportions of oligomers increased slightly with increasing reaction temperature and decreased with increasing concentration of hydroperoxide. This suggested that the decomposition of hydroperoxides proceeds mainly by a monomolecular homolytic cleavage (ROOH $\longrightarrow$ RO. + .OH). At higher temperatures, however, the decomposition of hydroperoxides also proceeds by the formation of esters with fatty acid. These compounds can then react with other fatty acid molecules to form di-esters and higher molecular mass products as shown in Figure 2. This mechanism would suggest the presence of C-O-C bonding between fatty acids in dimeric and trimeric structures formed by this route at temperatures between 60°C to 120°C.

It is generally believed that dimers form under autoxidative conditions only after considerable degradation has occurred (Chang and Kummerow, 1953), or when heat is applied. Miyashita et al., (1982) have shown, however, that dimers are formed from methyl linoleate during the initial stages of autoxidation (after 24 h at 30°C with aeration).

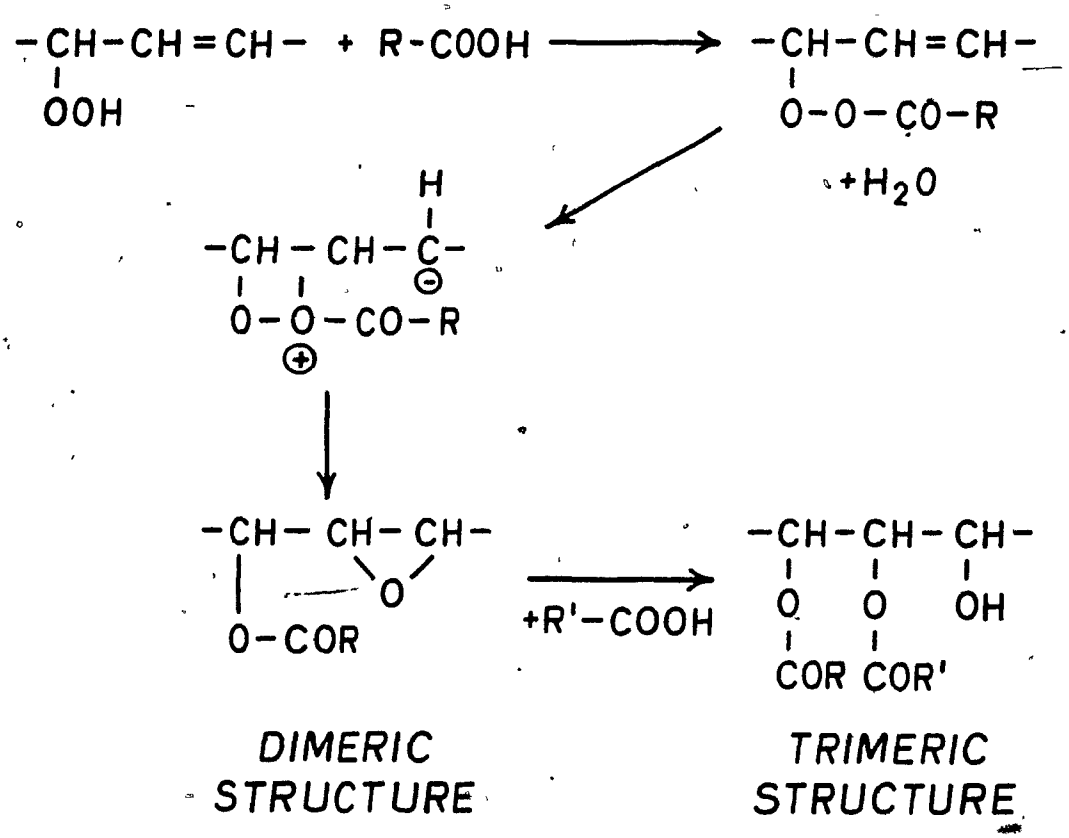


Fig. 2. The effect of the free carboxyl group on the decomposition of lipid hydroperoxides. (From Pokorny et al., 1976a)

Under these conditions, most of the dimers were linked through -C-O-O-C- bonds (peroxide linkages) and contained hydroperoxy and/or carbonyl groups and conjugated dienes. Thus dimers linked through peroxide linkages could be characteristic of autoxidized oils.

Perkins and Wantland (1973) subjected synthetic 1-linoleyl-2, 3-distearin to thermal oxidation (200°C, 24 h in the presence of air). They identified both noncyclic dehydrodimers and Diels-Alder dimers in the polar fraction.

Chang et al. (1978) used simulated deep-fat frying conditions in their study of the nonvolatile degradation products of pure trilinolein, triolein, and tristearin. After the heat treatment (185°C, 74 h with periodic injections of steam), trilinolein yielded 26.3% non-urea-adduct forming (NUAF) esters, triolein yielded 10.8% and tristearin yielded 4.2% of the NUAF esters. Further analysis indicated that polymers were formed from all three triglycerides. The polymers were essentially dimers and trimers; only trilinolein, however, yielded a fraction characterized as a cyclic dimer (Table 4). The study indicated that thermal oxidation of tristearin yielded dimers and trimers. There was also a change (0.0 to 0.5) in iodine value and this indicated that some dehydrogenation of fatty acids occurred.

Table 4. Estimated Composition of Oxidized and Polymerized Materials Formed During Simulated Deep Fat Frying at 185°C for 74 h (Chang et al., 1978)

Structure	Trilinolein (%)	Triolein (%)	Tristearin (%)
Cyclic dimers joined by C-C bonds	4.90	0.00	0.00
Non-cyclic dimers joined by C-C bonds	2.80	3.40	0.70
Trimers joined by two C-C bonds	8.40	0.30	0.36
Dimers or trimers joined wholly or partly by C-O linkages	4.90	6.20	1.20

Although most studies have concentrated on the degradation of long chain unsaturated acids, a few reports have been published on the thermal oxidation of short chain mono-unsaturated acids; interest in these acids stems from their potential importance as intermediate products in frying oils which themselves can undergo further degradation. Whitlock and Nawar (1976 a and b) presented some evidence for the presence of C-C linked dehydrodimers in the nonvolatile fraction obtained from ethyl 3-hexenoate, methyl 3-hexenoate, methyl 2-hexenoate, 3-hexenoic acid and 3-octenoic acid that had been subjected to thermal oxidation (150°C, 6 h, with aeration). The authors used a capillary column (152 m X 0.4 mm carbowax 20M) and were able to partially separate the dimer fraction into three

peaks, which were believed to be isomers of the same structure. The epoxy esters were another important group of compounds which was isolated only when the esters were degraded. The fact that they are very reactive and decompose readily, explains their absence among the products formed from the free acids.

Gilbert et al. (1981) demonstrated that epoxides also take part in dimerization reactions. The authors confirmed that dimeric epoxy fatty acid methyl esters were formed when epoxy acid methyl esters were heated (170°C for 5 h under N_2). In each experiment, the mass spectra suggested a mixture of four positional isomers, each containing an ether bridge linking a pair of fatty acid methyl esters across the carbon chains, with a keto group on a carbon adjacent to the bridge on one of the esters (Figure 3).

4.2 Cyclic Monomers

Cyclic monomers are an important class of nonvolatile thermal oxidation products because of their potential toxicity (Iwaoka and Perkins, 1976 and 1978). Among the many cyclic monomers which have been isolated from heated fats, the most common are the 18-carbon disubstituted cyclic fatty acids which have the general structure shown in Figure 4. A possible mechanism for the formation of this cyclic monomer from linolenic acid (Gente and Guillaumin, 1977) is given in Figure 5.

Michael (1966a) gave information on the isolation and characterization of aromatic cyclic monomers which were formed when methyl linoleate was heated (200°C, 200 h) in the presence of air. The aromatic products

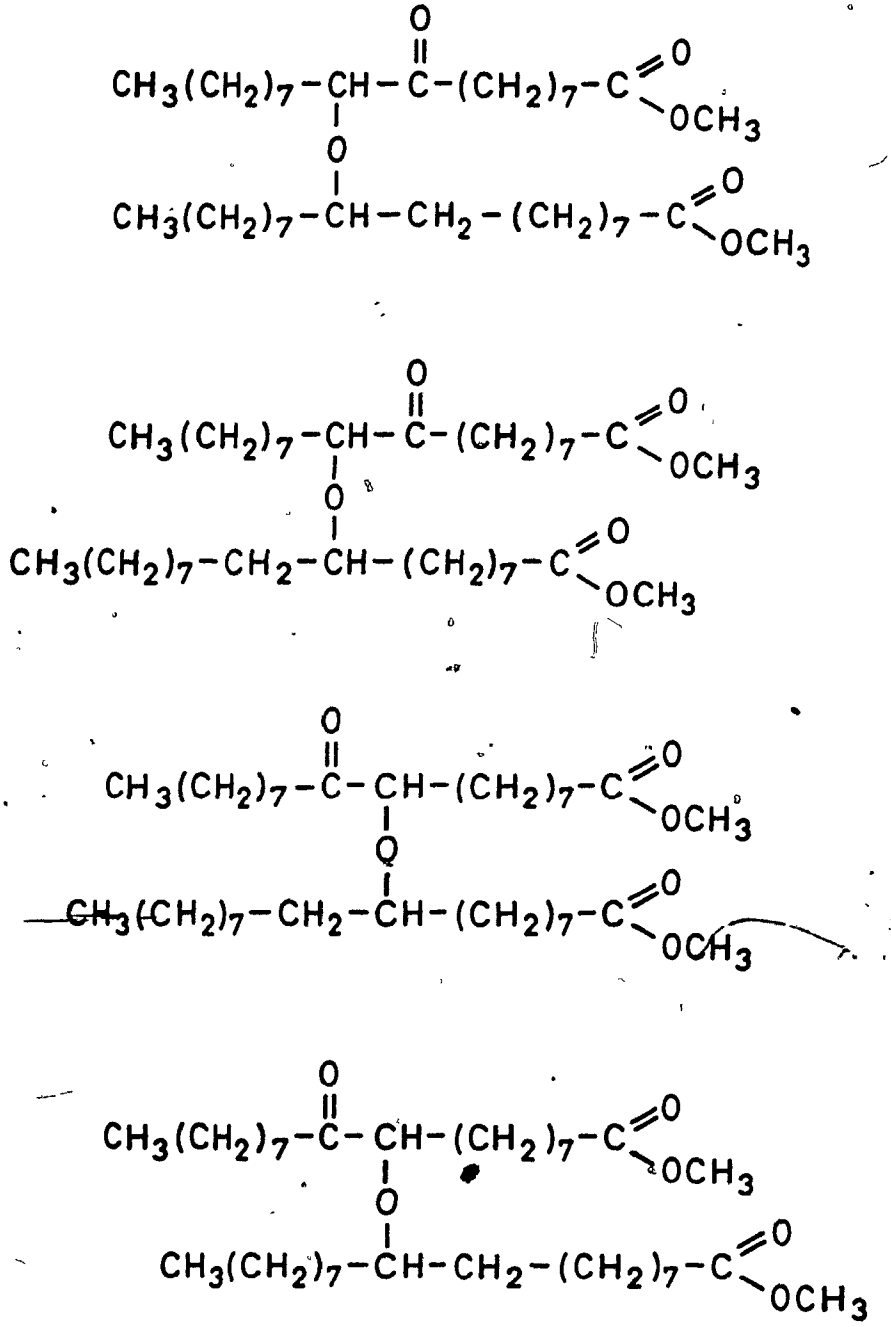


Fig. 3. Some possible isomeric structures for dimers formed by heating methyl 9, 10-epoxyoctadecanoate. (From Gilbert et al., 1981)

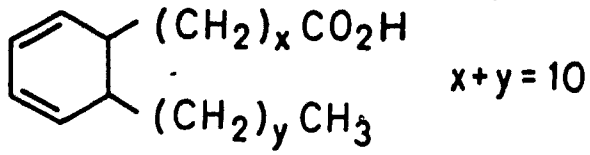


Fig. 4. A general structure for 18-carbon disubstituted fatty acids. (From Gente and Guillaumin, 1977)

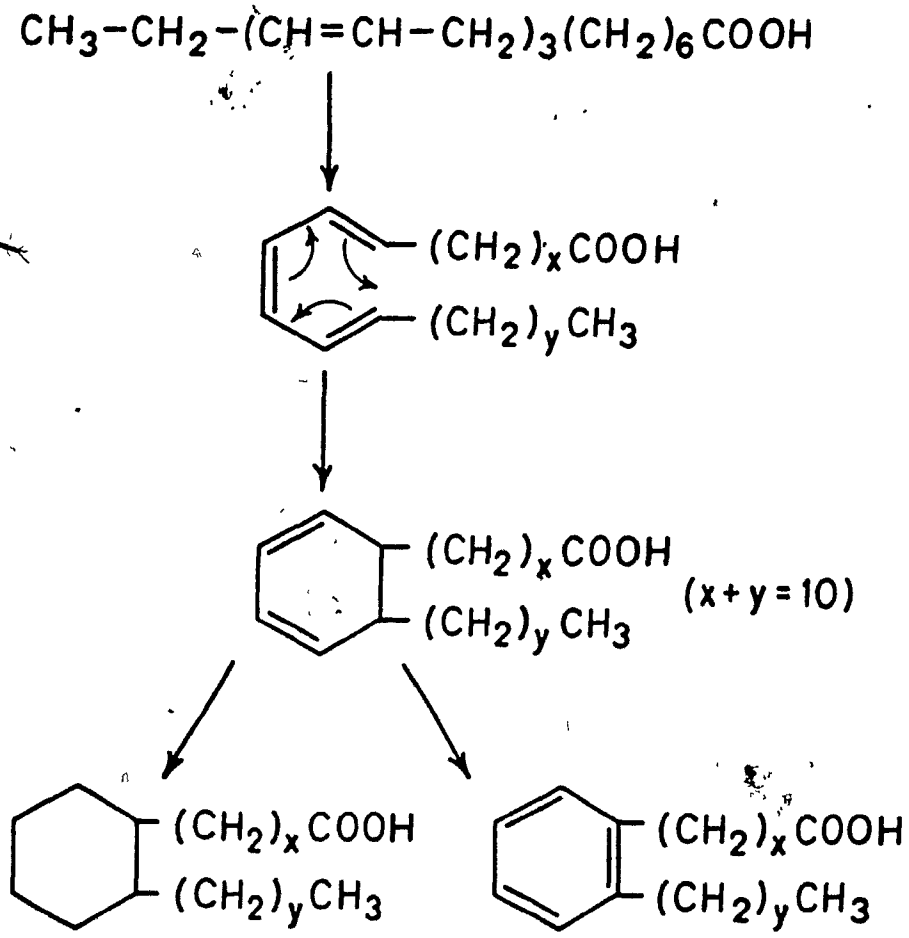


Fig. 5. A mechanism for the isomerization, cyclization and aromatization of linolenic acid. (From Gente and Guillaumin, 1977)

were shown to be methyl esters of ω (o-alkylphenyl) alkanoic acids containing 18 carbons. A possible mechanism for the formation of these aromatic cyclic esters (Figure 6) involves abstraction of hydrogen at allylic positions and intramolecular free radical to double bond addition.

Michael (1966b) proposed the structure shown in Figure 7 for a C18 nonaromatic cyclic monomer formed from methyl linoleate. A single mechanism to describe the formation of this compound has not been established; Michael, however, suggested a free radical allylic proton extraction, randomization of double bonds, and ring closure.

The formation of cyclic monomers from methyl oleate has not been reported (Paulose and Chang, 1978; Lercker et al., 1978). This might suggest that cyclic monomers are characteristic of dienoic and trienoic fatty acids only.

5. CHARACTERIZATION OF POLYMERS FROM THERMALLY OXIDIZED OILS

Naudet (1977) gave a quantitative estimate of the amounts of the various nonvolatile degradation products in thermally oxidized palm, peanut, soya, and sunflowerseed oils, although the heating conditions were not reported. Table 5 summarizes the results of this experiment.

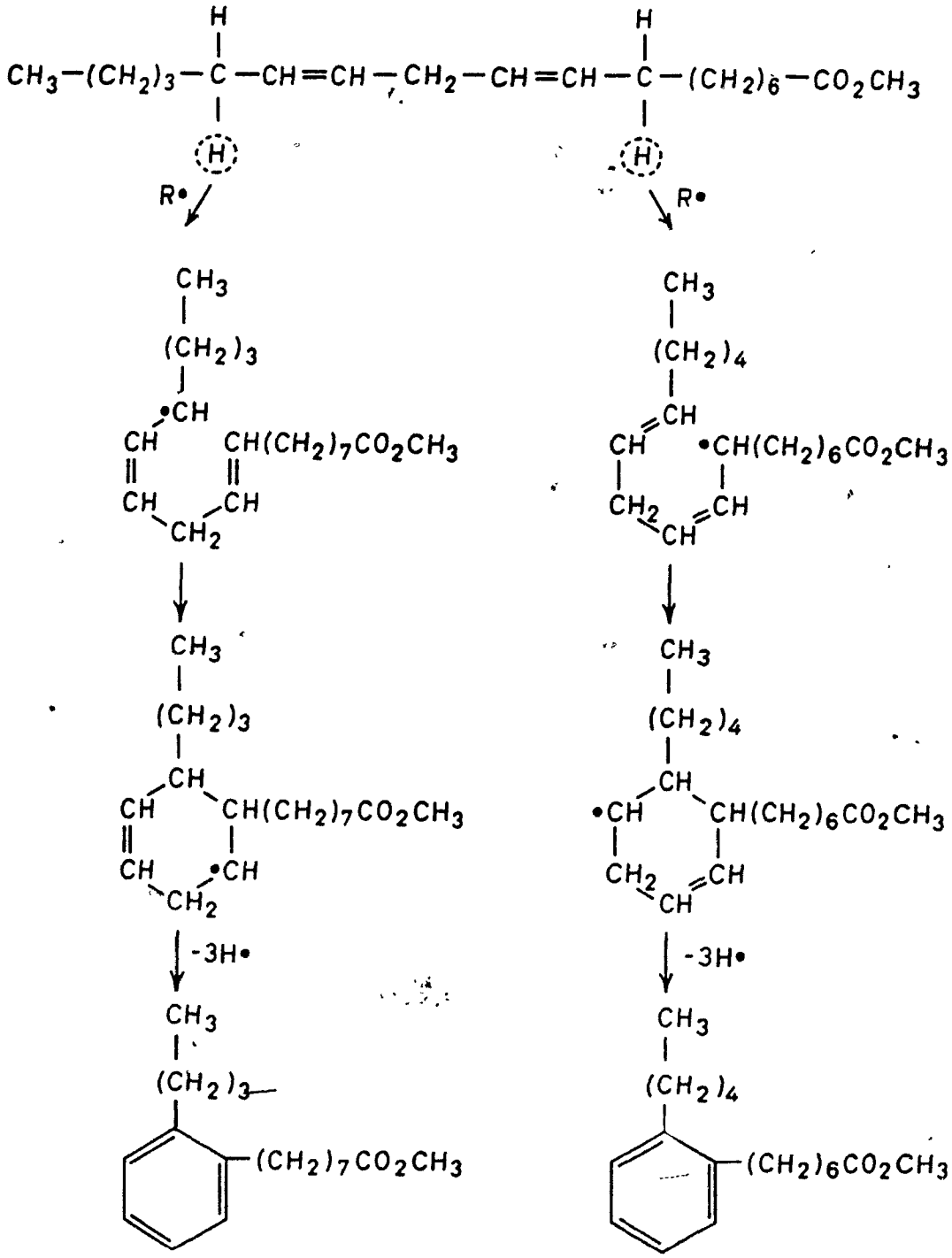


Fig. 6. A possible mechanism for the formation of aromatic C-18 esters from methyl linoleate. (From Michael, 1966a)

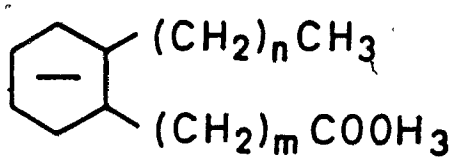
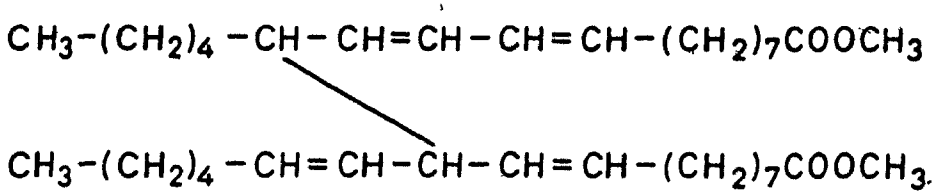


Fig. 7. A nonaromatic cyclic monomer, where $n = 2\text{-}5$, $m = 5\text{-}8$ and $n + m = 10$. The position of the double bond in the ring has not been defined. (From Michael, 1966b)

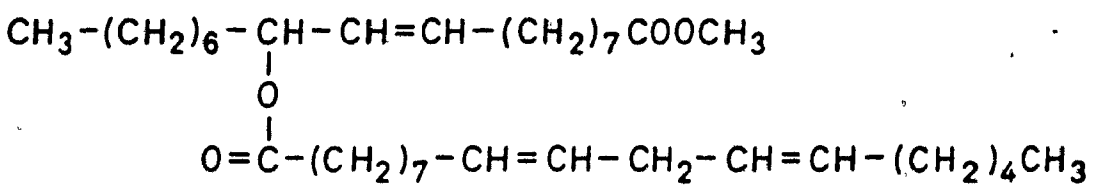
Table 5. Concentration (%) of Thermo-Oxidative Degradation Products in Four Oils (Naudet, 1977).

Structure	Oil			
	Palm	Peanut	Soya	Sunflower
Cyclic acids	0.15	0.15	0.20	0.15
Dimers	0.60	0.80	1.60	1.60
Estolides	0.40	0.80	0.30	0.40
Dimers (ether)	0.90	1.60	1.70	2.60
Oxymonomers	1.40	2.80	1.80	1.70
Oxypolymers	0.70	1.10	1.30	1.70

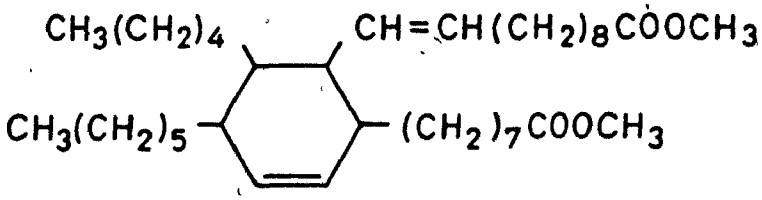
Ottaviani et al., (1979) and Naudet (1977) characterized the nonvolatile products which were formed in soybean oil which had been heated (220°C) in air for 30 min per day over 14 days. The methyl esters of the saponifiable products were separated into five fractions and characterized as follows: (i) nonpolar (85% to 95% of the total esters), including normal acids and small quantities of cyclic (saturated and unsaturated) aromatic acids; (ii) very slightly polar, including mainly dimers (cyclic and noncyclic) with C-C bonding, and estolides (Figure 8); (iii) slightly polar, including a complex mixture of monomers, dimers, and limited amounts of trimers and tetramers, all with the common characteristic of an ether bridge (Figure 9); (iv) very polar (normal molecular weight), including oxyacids and from monomers to tetramers containing hydroxyl, carbonyl, epoxide and peroxide functions, isolated or associated; (v) very polar (elevated molecular weight), including primarily oxypolymers with C-C, ether, peroxide, and epoxide bonding.



(a)

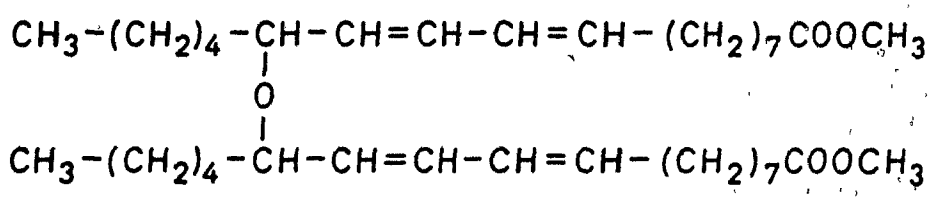


(b)

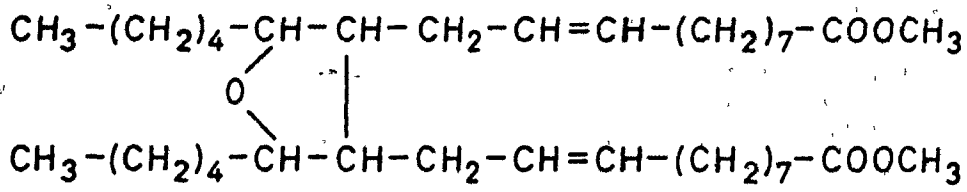


(c)

Fig. 8. Non-cyclic (a), estolide (b) and cyclic (c) dimer structures. (From Ottaviani et al., 1979)



(a)



(b)

Fig. 9. Dimer structure with an ether bridge (a) and a tetrahydrofuran cyclic dimer (b). (From Ottaviani et al., 1979)

Gente and Guillaumin (1977) found that the amount of cyclic monomers formed in heated oils depended on the linolenic acid content, temperature, and atmospheric conditions. Heated oils (240°C, 10 h in air) which were low in linolenic acid (less than 20%) contained 0.2 - 0.3% cyclic monomers. When the oils were heated at 275°C under nitrogen, the amount of cyclic monomers increased substantially. Linseed oil (linolenic acid content of 55.2%) contained 0.9% cyclic monomers when it was heated at 220°C in air for 20 h, 1.4% cyclic monomers when it was heated at 240°C in air for 10 h, and 4.95% cyclic monomers when it was heated at 275°C under N_2 for 12 h. Meltzer et al. (1981) detected cyclic acids (0.3% to 0.6%) in heated (195°C for 52 - 104 h) soybean oil under both continuous and intermittent heating conditions. Fresh oils, prior to heating, have been shown to contain very small amounts of cyclic monomers (0.1% to 0.2%), probably as a result of previous processing and refinement (Guillaumin et al., 1977). In a recent study by Frankel et al. (1984), vegetable fat and oil samples from food outlets in both the United States and the Middle East (Israel and Egypt) were analyzed for their content of cyclic fatty acid monomers. The samples from the USA contained from 0.1% to 0.5% cyclic monomers and from 1% to 8% polar plus noneluted materials; the samples from Israel and Egypt had values for cyclic monomers ranging from 0.2% to 0.7% and polar materials ranging from 2% to 22%.

6. THERMAL OXIDATION OF SATURATED FATTY ACIDS

Saturated fatty acids or their esters are relatively stable to oxidation at low temperatures (less than 60°C). As the temperature is increased to 150°C, oxidation occurs but at a slower rate than with unsaturated fatty acids (Brodnitz et al., 1968a). Consequently, thermal oxidation of saturated fatty acids has not been studied as extensively as have the mono and polyunsaturated fatty acids. The papers which have appeared in the literature have reported mainly on the volatile degradation products.

Chang et al. (1978) provided some evidence for the presence of polymeric compounds in pure systems of saturated fatty acids which were subjected to thermal oxidation. The authors reported that noncyclic dimers which contained C-C bonds (0.70%), dimers and trimers which contained C-O linkages (1.2%), and trimers which contained C-C linkages (0.36%), were formed when tristearin was heated under simulated deep fat frying conditions (185°C, 75 h, periodic injections of steam).

It has been suggested that the introduction of unsaturation into the saturated fatty acid molecule by dehydrogenation is the first step in the thermal oxidative deterioration of saturated fatty acids (Ramanathan et al., 1959; Endres et al., 1962; Chang et al., 1978). This was evidenced by an increase in iodine value and evolution of hydrogen during thermal oxidation. Subsequent attack by oxygen at the double bond, formation of hydroperoxides and decomposition is believed to follow the same route as it does with monounsaturated acids.

Crossley et al. (1962) and Endres et al. (1962) reported that the major oxidative products of saturated triglycerides include a homologous series of carboxylic acids, methyl ketones and alkanals. Selke et al. (1975) added alkanes, alkenes, alcohols and gammalactones to this list.

There is a difference of opinion among different investigators as to the preferential position of oxygen attack. Although oxygen attack can occur at all of the methylene groups of the fatty acid, several workers have suggested that oxidation occurs preferentially at the positions where the fatty acids are linked to the glycerol backbone (Crossley et al., 1962; Endres et al., 1962; Jewell and Nawar, 1980) since the dominant oxidative products had chain lengths near or equal to the parent fatty acids. Conversely, Brodnitz et al. (1968 a and b) and Selke et al. (1975) suggested that oxygen attack toward the center of the molecule was favoured. Ramanathan et al. (1959) observed that methyl stearate was more susceptible to oxygen attack than was methyl laurate. This suggests that the chain length of saturated fatty acids influences their susceptibility to oxidation.

Endres et al. (1962) showed that hydrolysis of the ester link in the triglyceride molecule occurs during thermal oxidation of saturated fatty acids; this may be followed by oxygen attack on the products of hydrolysis as shown above. Noble et al. (1967) noted a trend towards selectivity of hydrolysis in favour of the shorter chain and unsaturated acids.

Witchwoot et al. (1981) noted that when trilaurin, tricaprin or trimyristin were heated in the presence of ethyl linoleate, the volatile degradation products contained no volatiles which could be ascribed to the saturated triglycerides. A similar observation was made by Selke et al. (1980) when mixtures of tristearin and trilinolein were thermally oxidized. These results suggest that unsaturated fatty acids inhibit the oxidation of saturated chains; this could be because free radicals are more likely to abstract hydrogen preferentially from the pentadiene system of linoleate. This inhibition by linoleate could be limited to oxidative degradation as noted by Crnjar et al. (1981) who reported that the presence of unsaturation did not have a marked influence on nonoxidative reactions of the saturated system. Selke et al. (1977) also reported a difference between polyunsaturation and monounsaturation. When tristearin was heated with triolein, decomposition products of stearate were detected.

CHAPTER III

PHYSICAL AND CHEMICAL CHARACTERISTICS OF BUTTERFAT FRACTIONS OBTAINED BY CRYSTALLIZATION FROM MOLTEN FAT

1. INTRODUCTION

Among all of the naturally occurring fats, butterfat is the most varied in its chemical and physical characteristics. Patton and Jensen (1975) listed the presence of 437 different fatty acids in butterfat; they comprised normal, iso and anteiso monobranched and multibranched saturates, cis- and trans-monoenes, dienes, polyenes, keto, hydroxy and cyclic fatty acids. Because of this great variety of fatty acids, a large number of different triglycerides can be formed; consequently, the melting and crystallization of butterfat occurs over a wide temperature range. The unique character of butterfat, however, can be considered to be a shortcoming when compared to the wide range of specialty vegetable fats and oils which are available to the food-fat industry. One way to over-come this shortcoming is to modify the butterfat by fractionation to produce products which differ markedly from one another in chemical and physical characteristics and which are suitable for use in specific food industries. Proposed food applications for butterfat fractions have included the following: (i) the incorporation of a low-melting fraction into milk powder to improve reconstitutibility (Baker et al., 1959; Schaap and Van Beresteyn, 1972); (ii) the use of low-melting fractions to

make normal butter softer (McGillivray, 1972; Larsen and Samuelsson, 1979); (iii) the use of high-melting fractions as shortenings for puff pastry and french rolls (Schaap and Kim, 1981); and (iv) the substitution of cocoa butter in confectionary products by high-melting fractions of butterfat (McGillivray, 1972).

Fractionation of butterfat can be made either by crystallization of the fat dissolved in an organic solvent such as acetone or alcohol (Chen and de Man, 1966; Colombini et al., 1979; Larsen and Samuelsson, 1979) or by direct cooling of the melted fat followed by separation of the crystalline matter from the liquid oil by filtration or centrifugation (de Man, 1968; Black, 1973 and 1975; Schaap and Rutten, 1976; de Man and Finoro, 1980; Badings et al., 1983 a and b). Although recrystallization from solvents yields a more distinct separation between crystalline and liquid fractions, the latter method is preferred for food industry applications because of (a) flavour and toxicological problems that may result from solvent residues; (b) the high cost of solvent recovery, and (c) the possibility of destruction of desirable aroma components and vitamins. The main technological problem in the fractionation of butterfat without the use of solvents has been the separation of the crystals from the liquid fraction. The cooling temperature and the rate of crystallization strongly influence the composition, quantity and size of the fat crystals. Several investigators (de Man, 1968; Black, 1975; Schaap and Rutten, 1976) have shown that large crystals which are obtained by a slow cooling rate and with slow agitation are more easily filtered .

The products which were obtained in experiments involving butterfat fractionation using molten fat have been characterized (de Man and

Finoro, 1980; Badings et al., 1983 a and b). The procedures which were used, however, would be impractical on an industrial scale. This study was undertaken to characterize butterfat fractions which were obtained in the laboratory using a procedure which closely resembles that used in the Tirtiaux industrial fractionation process (S.A. Fractionnement Tirtiaux, Belgium). The Tirtiaux process is based on a slow controlled cooling of the oil, a short stabilization time at the fractionation temperature followed by separation of the crystals on a continuous vacuum filter equipped with a stainless steel perforated belt as the filtration support (Tirtiaux, 1983). Characterization of the chemical and physical properties of fractions obtained by this procedure should aid in defining possible uses of these fractions in the food industry.

Both summer and winter butterfats have been fractionated. The results of analyses (fatty acid and triglyceride analyses, thermal examinations by differential scanning calorimetry, melting point, iodine value and peroxide value) of the various fractions are reported in this chapter. The thermal oxidative stability of these fractions in comparison to those of selected vegetable oils will be reported in subsequent chapters.

2. EXPERIMENTAL

2.1 Anhydrous Butterfat

Anhydrous butterfat was prepared from fresh butter (Cooperative Agricole de la Cote Sud, Quebec) by melting the butter at 60°C, removing

the top oil layer, filtering the oil through glass wool and drying the resulting product over anhydrous sodium sulfate. The oil was then refiltered (vacuum, Whatman 41 paper), flushed with nitrogen and stored at -20°C until it was fractionated.

2.2 Fractionation Procedure

Anhydrous butterfat was fractionated according to a standardized batch procedure at 29, 26, 23 and 19°C for winter (January) butterfat and 29 and 19°C for summer (September) butterfat. Preliminary experiments indicated that at temperatures above 29°C, the yields of solid fat were very small; hence, the products were not used in the present study. Below 19°C for winter butterfat and 17°C for summer butterfat, the solid fraction was massive and the liquid fraction could not be separated readily.

The crystallization equipment consisted of a Hobart mixer equipped with a flat beater attachment and a bowl (12 l capacity) which was modified to include a water jacket. Temperature control of the bowl was accomplished by using a Haake D1 circulating water bath and an EK 12 cooling unit. Both the bowl and the butterfat were preheated to 60°C and then 1.5 l of the molten butterfat was transferred to the bowl. As soon as the oil was added, the cooling unit was turned on and the temperature control of the circulating water bath was adjusted to 2-3°C below the fractionation temperature. The oil was cooled slowly to the fractionation temperature with continuous agitation (20 rpm). The length of the cooling period varied depending on the final fractionation

temperature, the proportion of crystallizable fat and the heat of crystallization. After 4 hours of total cooling and heating time, the solid crystals were separated from the liquid oil by filtration (14 mesh stainless steel filter; Tirtiaux, Belgium) using vacuum (50-100 mbar below atmospheric pressure). The solid and liquid fractions were weighed and the percentage yield of each fraction was calculated. The fractionation procedure was replicated three times at each temperature.

2.3 Fatty Acid Analysis

The fatty acid composition of each fraction was determined after conversion of the fatty acids into the corresponding methyl esters by a modification of the method of Christopherson and Glass (1969). A portion of the anhydrous lipid sample (200 mg) was placed in a vial (7 ml), and petroleum ether (2 ml) was added to dissolve the sample. Sodium methoxide solution (0.1 ml; 2 N $NaOCH_3$ in anhydrous methanol) was added, and the contents of the vial were mixed (1 min) using a vortex mixer. After sedimentation of the sodium glycerolate, a portion of the clear supernatant was dissolved in hexane (1 part supernatant to 100 parts hexane). An aliquot (0.2 µl) of the hexane solution was injected into a fused silica capillary column (30 m x 0.32 mm ID) coated with SP-2340 (Supelco Inc., Canada) (0.2 µm). The column was placed into a Varian 3700 gas chromatograph equipped with a flame ionization detector and a cold on-column injector. The oven temperature was programmed from 50°C to 160°C (5°C/min) after a 1-min hold at 50°C. The carrier gas (helium)

flow rate was 1.5 ml/min. The injector temperature was programmed from 70°C to 200°C (100°C/min), and the detector temperature was maintained at 210°C. Correction factors were determined by analysis of a standard mixture of fatty acid methyl esters (Nu Chek Prep, Elysian, Minnesota) having a composition which resembled that of an average butterfat sample.

2.4 Triglyceride Analysis

The triglyceride composition was determined by dissolving approximately 15 mg of the anhydrous butterfat or butterfat fraction in 6 ml of hexane and injecting an aliquot (0.2 µl) into a bonded phase fused silica capillary column (DB-5, 0.1 µm; 15 m x 0.32 mm ID; J&W Scientific Inc., Rancho Cordova, California). The same gas chromatograph was used as for fatty acid analyses. The oven temperature was programmed in two stages as follows: first, from 50°C to 240°C at a rate of 25°C/min, and then from 240°C to 350°C at a rate of 3°C/min. The injector temperature was programmed from 70°C to 330°C (100°C/min), and the detector temperature was maintained at 350°C. The carrier gas was helium (1.5 ml/min). Identification of the major groups of triglycerides according to carbon number was made by comparison of retention times to those of standard mixtures of simple triglycerides from C18 to C54 (Nu Chek Prep, Elysian, Minnesota).

2,5 Differential Scanning Calorimetry (DSC)

Crystallization and melting curves were recorded using a heat flow differential scanning calorimeter (Mettler TA 3000, Mettler Instrument Ltd., Switzerland), calibrated with indium. A sample (20-30 mg) of fat was placed in an aluminum crucible; the crucible was covered with a pierced lid and sealed. The measuring cell was purged with nitrogen gas (50 ml/min) during the analysis. The samples were treated as follows: (i) heating at 80°C for 10 min to destroy the structure that was associated with the previous thermal history; (ii) crystallization over the temperature range 80°C to -40°C (rate of cooling, 10°C/min, using liquid air); (iii) fusion of the crystals formed by heating over the temperature range -40°C to 80°C (rate of heating, 10°C/min). The relative percentages of liquid fat as a function of temperature were determined by integration of the DSC melting curve of the fat sample using a Mettler TC 10 data processor.

2.6 Iodine Value

Iodine values were determined by the Cd 1-25 method of the American Oil Chemists' Society (AOCS, 1980). The titrations were performed by the use of a Mettler DL 40 RC Memo Titrator (Mettler Instrument Ltd., Switzerland).

2.7 Melting Point

Melting points were determined by the Cc 1-25 method of the American Oil Chemists' Society (AOCS, 1980).

2.8 Peroxide Value

Peroxide values were determined by the Cd 8-53 method of the American Oil Chemists' Society (AOCS, 1980).

2.9 Statistical Analysis

Analyses of variance were performed on the mean values (3 replications x 2 duplicates) of the following: major groupings of fatty acids; iodine value data; melting point data and percentage liquid fraction data. Where significant differences were noted, Duncan's Multiple Range test was used to determine which samples were significantly different. The analyses were performed using the McGill University System for Interactive Computing (MUSIC) and the Statistical Analysis System (SAS).

3. RESULTS AND DISCUSSION

3.1 Yield of Solid Fractions and Percentage of Liquid Oil in the Fractions

Table 6 shows the results of two experiments (three replications) in which fat obtained from butter produced (a) during the winter months and (b) during the summer months was fractionated. The winter butterfat was fractionated at 29, 26, 23 and 19°C to obtain a solid (S) and liquid (L) fraction at each temperature; summer butterfat was fractionated at 29 and 19°C. The largest variations in yield of solid between replicate runs were observed for the 29°C winter and summer fractions and the 23°C winter fractions. These differences could be related to differences in the amounts of liquid oil in the solid fractions (Table 6).

The percentage of liquid oil in the solid and liquid fractions was determined by differential scanning calorimetry (DSC). The values for the solid fractions ranged from 38.2% to 52.3% for the fat from winter butter and from 36.2% to 48.8% for the fat from summer butter (Table 6). Results from the corresponding liquid fractions showed that they contained approximately 2-6% of solid contaminants (Table 6). These could arise from resolubilization of solid crystals during the filtration step which was conducted at room temperature. Badings et al. (1983 a and b) measured the percentage of liquid oil in solid fractions obtained by stepwise crystallization of melted butterfat. They reported values ranging from 63.4% to 83.8% based on the distribution of carotene between the cake and the filtrate.

Table 6. Results of Butterfat Fractionation By Cooling Liquid Fat.

		Replication 1		Replication 2		Replication 3	
Fraction[1]	Fractionation temperature (°C)	Yield (%) solid fraction	Composition[2] (% liquid in fraction)	Yield (%) solid fraction	Composition (% liquid in fraction)	Yield (%) solid fraction	Composition (% liquid in fraction
				Fat from winter butter			
S-29	29	16.2	38.2	22.0	44.4	23.7	49.2
L-29	29	-	95.5	-	97.5	-	97.2
S-26	26	34.3	46.4	37.4	50.9	36.9	50.0
L-26	26	-	96.3	-	96.8	-	96.2
S-23	23	60.0	52.3	55.9	51.9	52.2	50.2
L-23	23	-	97.8	-	98.0	-	97.7
S-19	19	62.6	48.8	60.8	47.8	61.7	47.7
L-19	19	-	95.0	-	95.0	-	93.2
				Fat from summer butter			
S-29	29	16.1	41.6	13.6	36.2	15.0	41.6
L-29	29	-	96.2	-	96.8	-	96.6
S-19	19	53.9	48.8	53.9	48.4	53.4	48.4
L-19	19	-	95.4	-	93.6	-	93.8

[1] Fraction designation indicates physical state (solid, liquid) and fractionation temperature.
[2] The % liquid in fraction was determined by Differential Scanning Calorimetry (DSC).

3.2 Chemical Composition of Fractions

Table 7 shows that the iodine values varied from 24.70 to 33.07 for the winter fractions and from 29.18 to 37.67 for the summer fractions. This variation was greater than the variation in iodine value of the whole fats from winter and summer butter (30.26 and 35.78 respectively). With the exception of fractions S-19 and S-23 (fat from winter butter), the differences in iodine value between the various fractions and the whole butterfat from each season were significant ($p<0.05$).

The peroxide values of the whole butterfat and the liquid fractions obtained from this fat were measured to evaluate the effect of the various processing steps (separation of butterfat from butter, storage, re-melting, crystallization and filtration) on the oxidative stability of the fractions. In all instances, the peroxide values were found to be 0.0 meq O_2/kg fat.

The fatty acid compositions of the whole fats (winter and summer) and their corresponding fractions are summarized in Tables 8, 9, 10 and 11. For simplicity of presentation and statistical analyses, the fatty acids (about 40) which were determined have been grouped according to similarities in melting behaviour. Unidentified peaks were combined in the "other" category. It is noteworthy to mention that the procedure used for fatty acid composition allowed for the determination of some positional and geometric isomers of unsaturated fatty acids; in particular, elaidic acid and both cis and trans vaccenic acid were identified.

Table 7. Iodine Values of Whole Butterfat and Butterfat Fractions.

Fraction	Iodine Value	
	Experiment 1 (fat from winter butter)[1]	Experiment 2 (fat from summer butter)[1]
S-29	24.70[h] (±0.95)	29.18[e] (±0.33)
S-26	26.70[g] (±0.38)	-
S-23	27.78[f] (±0.25)	-
S-19	27.94[f] (±0.37)	32.80[d] (±0.18)
Whole butterfat	30.26[e]	35.78[c]
L-29	31.44[d] (±0.06)	36.13[b] (±0.09)
L-26	31.95[c] (±0.05)	-
L-23	32.70[b] (±0.12)	-
L-19	33.07[a] (±0.24)	37.67[a] (±0.02)

[1] Fractionation experiments were performed in triplicate at each temperature; the analysis of each sample was performed in duplicate. Means in each column with different letter superscripts are significantly different at the 5% probability level. The deviation is the average deviation from the mean of three replicate fractions, analyzed in duplicate.

From the statistical analyses which were performed on the fatty acid data (winter fractions; Tables 8 and 9), it will be noted that with the short chain saturates, medium chain saturates, long chain saturates and cis unsaturates, the 29 and 19°C fractions (with the exception of fraction L-29 vs whole butterfat) are significantly different ($p<0.05$) from each other and from the whole butterfat. This was not necessarily true for the 26 and 23°C fractions. In all categories, the S- and L-23 fractions were not significantly different ($p<0.05$) from the S- and L-19 fractions, respectively. Similarly, the L-26 fractions were not significantly different from the L-29 fractions.

It will be noted from the results obtained with the fractions of summer butterfat (Tables 10 and 11) that the 29 and 19°C fractions were significantly different ($p<0.05$) from each other and from the whole butterfat with respect to short chain saturates, long chain saturates and cis unsaturates.

In general, the short chain saturated fatty acids (C4:0 to C10:0) were found in greater amounts in the liquid fractions, and the long chain saturated fatty acids (C16:1 to C20:0) were found in greater amounts in the solid fractions. The cis unsaturated fatty acids (C10:1 to C18:3) followed the same trend as the short chain saturated fatty acids. This reflected the similarities in melting behaviour of these two groups of fatty acids. As the fractionation temperature decreased, the concentration of short chain saturated fatty acids and cis unsaturated fatty acids in the liquid fractions increased. The trans 18:1 isomer, however, showed the reverse tendency. The trans 18:1 fatty acids, as a

Table 8. Fatty Acid Composition of Winter Butterfat and Solid Butterfat Fractions.

Fatty acids	Methyl esters (Wt %)[1] S-29	S-26	S-23	S-19	Whole winter butterfat
Short chain, saturated (C4:0 to C10:0)	10.40^{d}	11.47^{c}	12.08^{b}	12.17^{b}	12.97^{a}
Medium chain, saturated (C12:0 to C15:0)	17.52^{a}	17.27^{a,b}	17.24^{a,b}	17.07^{b,c}	16.86^{c}
Long chain, saturated (C16:0 to C20:0)	47.38^{a}	44.83^{b}	43.53^{c}	42.90^{c}	40.65^{d}
cis, unsaturated (C10:1 to C18:3)	20.43^{d}	21.96^{c}	22.93^{b}	23.18^{b}	24.92^{a}
trans, unsaturated (C18:1)	1.46^{a}	1.43^{a}	1.47^{a}	1.55^{a}	1.55^{a}
Other	2.81^{a}	3.04^{a}	2.74^{a}	3.13^{a}	3.04^{a}
$\frac{\text{trans 18:1}}{\text{Total 18:1}} \times 100$	8.65^{a}	7.97^{a,b}	7.81^{a,b}	8.14^{a,b}	7.58^{b}

[1] Fractionation experiments were performed in triplicate at each temperature, the analysis of each sample was performed in duplicate. Means in each row with different letter superscripts are significantly different at the 5% probability level.

Table 9. Fatty Acid Composition of Winter Butterfat and Liquid Butterfat Fractions.

Fatty acids	Methyl esters (Wt %)[1]				
	Whole winter butterfat	L-29	L-26	L-23	L-19
Short chain, saturated (C4:0 to C10:0)	12.97^{d}	13.34^{c,d}	13.77^{b,c}	14.29^{a,b}	14.84^{a}
Medium chain, saturated (C12:0 to C15:0)	16.86^{a}	16.49^{b}	16.46^{b}	16.23^{b,c}	16.09^{c}
Long chain, saturated (C16:0 to C20:0)	40.65^{a}	38.89^{b}	38.49^{b}	37.50^{c}	36.69^{d}
cis, unsaturated (C10:1 to C18:3)	24.92^{d}	26.50^{c}	26.89^{b}	27.61^{a}	27.72^{a}
trans, unsaturated (C18:1)	1.55^{a,b}	1.64^{a}	1.30^{b}	1.36^{a,b}	1.40^{a,b}
Other	3.04^{a}	3.12^{a}	3.12^{a}	3.01^{a}	3.25^{a}
$\frac{\text{trans 18:1}}{\text{Total 18:1}} \times 100$	7.58^{a}	7.52^{a}	5.95^{b}	6.05^{b}	6.23^{b}

[1] See Table 8

Table 10. Fatty Acid Composition of Summer Butterfat and Solid Butterfat Fractions.

Fatty acids	Methyl esters (Wt %)[1] S-29	S-19	Whole summer butterfat
Short chain, saturated (C4:0 to C10:0)	9.37[c]	11.10[b]	11.82[a]
Medium chain, saturated (C12:0 to C15:0)	15.88[a]	15.78[a]	15.44[b]
Long chain, saturated (C16:0 to C20:0)	47.04[a]	41.75[b]	39.47[c]
cis, unsaturated (C10:1 to C18:3)	21.14[c]	24.73[b]	26.37[a]
trans, unsaturated (C18:1)	3.13[a]	3.06[a]	3.35[a]
Other	3.44[a]	3.58[a]	3.55[a]
$\frac{\text{trans 18:1}}{\text{Total 18:1}} \times 100$	16.13[a]	13.76[b]	14.06[b]

[1] See Table 8

Table 11. Fatty Acid Composition of Summer Butterfat and Liquid Butterfat Fractions.

Fatty acids	Methyl esters (Wt %)[1]		
	Whole summer butterfat	L-29	L-19
Short chain, saturated (C4:0 to C10:0)	11.82[c]	12.95[b]	13.99[a]
Medium chain, saturated (C12:0 to C15:0)	15.44[a]	15.26[a,b]	15.02[b]
Long chain, saturated (C16:0 to C20:0)	39.47[a]	36.98[b]	35.68[c]
cis, unsaturated (C10:1 to C18:3)	26.37[c]	27.81[b]	29.22[a]
trans, unsaturated (C18:1)	3.35[a]	3.15[a]	2.55[b]
Other	3.55[a]	3.84[a]	3.55[a]
$\frac{\text{trans 18:1}}{\text{Total 18:1}} \times 100$	14.06[a]	12.78[b]	10.10[c]

[1] See Table 8.

proportion of the total octadecenoic acids, were significantly higher ($p<0.05$) in the S-29 fractions and significantly lower ($p<0.05$) in the L-19 fractions compared to the original butterfats. The trans 18:1 isomers consisted mainly of the Δ 11 isomer (mp 44°C) while the cis 18:1 is mainly the Δ9 isomer (mp 11°C). This accounts for the occurrence of a higher proportion of the trans 18:1 in the solid than in the liquid fractions.

The present study shows more marked differences in fatty acid composition between fractions obtained by cooling liquid fat than have been previously reported (Norris et al., 1971; Black, 1973; de Man and Finoro, 1980). The improved crystallization and separation procedures used in the present study permitted a wider range of fractionation temperatures to be used and hence larger differences in chemical composition of fractions compared to those reported previously.

In addition to the fatty acid analyses, the whole butterfat and butterfat fractions from the winter butterfat were analyzed by capillary column gas chromatography (CC-GC) for triglyceride composition. Grob and coworkers (1980) noted that fat fractionation can be monitored more sensitively on a triglyceride basis than by classical analysis of fatty acid methyl esters; this is because it is a direct analysis of glyceride species that are being separated. Figure 10 shows typical chromatograms (CC-GC method) which were obtained with winter butterfat and with S-29 and L-19 fractions. The triglyceride compositions according to acyl carbon number, for both solid and liquid, 29 and 19°C winter fractions in comparison to the whole fat, are summarized in Table 12.

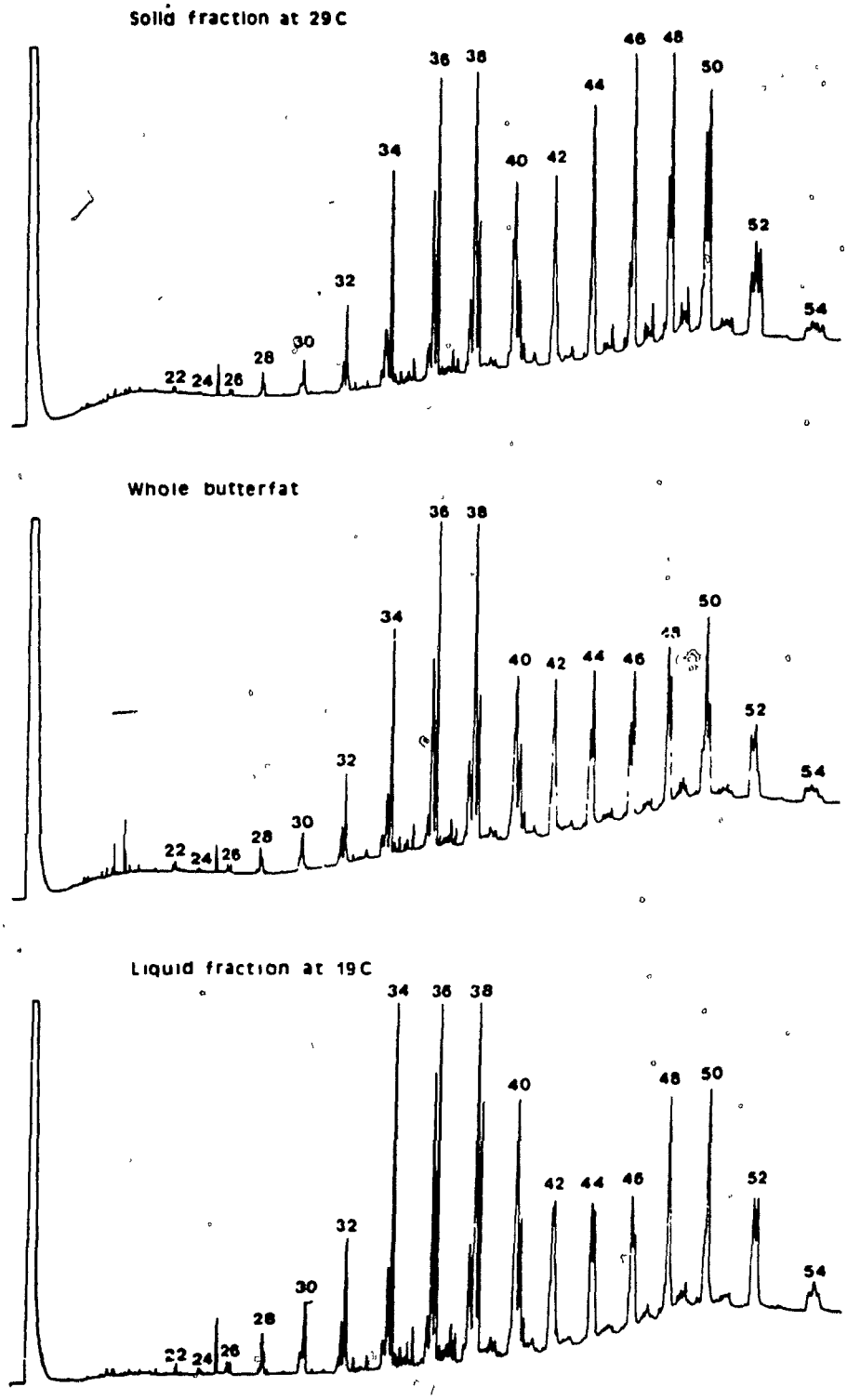


Fig. 10. Typical gas chromatograms of the triglycerides of whole butterfat, a solid fraction at 29°C, and a liquid fraction at 19°C.

Table 12. Triglyceride Composition of Winter Butterfat and Butterfat Fractions as Determined by Gas Chromatography.

Acyl carbon number	% of Triglyceride (based on peak area/total area of all peaks)				
	S-29[1]	S-19[1]	Whole winter butterfat[2]	L-29[1]	L-19[1]
22	tr[3]	0.06	tr	0.08	0.10
24	tr	0.03	tr	0.08	0.08
26	0.11	0.14	0.16	0.17	0.18
28	0.38	0.57	0.52	0.68	0.79
30	0.82	1.04	1.18	1.25	1.46
32	1.73	2.18	2.32	2.57	2.84
Other	0.22	0.27	0.29	0.30	0.34
34	4.76	5.77	6.58	6.66	7.52
Other	0.95	1.08	1.36	1.31	1.44
36	9.12	10.91	11.35	12.50	14.03
Other	1.29	1.55	1.52	1.71	1.99
38	10.12	12.09	13.67	13.74	15.44
Other	0.41	0.65	0.69	0.71	0.81
40	7.82	9.33	10.17	10.33	11.31
Other	0.23	0.50	0.12	0.50	0.58
42	6.50	7.14	7.29	7.05	6.82
Other	0.28	0.47	0.72	0.46	0.31
44	7.32	6.96	6.58	5.90	5.37
Other	0.95	0.90	0.79	0.63	0.27
46	9.08	7.55	6.60	5.92	5.03
Other	1.45	1.14	0.90	0.95	0.39
48	10.96	8.87	8.12	7.23	6.15
Other	2.86	2.02	2.18	2.24	1.24
50	11.66	9.35	8.40	8.11	6.88
Other	1.84	0.97	0.77	1.12	0.70
52	7.04	6.41	6.12	5.78	5.73
54	2.09	2.07	1.62	2.04	2.20

[1] Fractionation experiments were performed in triplicate at each temperature; the analysis of each sample was performed in duplicate.

[2] The sample was analyzed in duplicate.

[3] Trace.

It will be noted from Figure 10 that the composite groups of peaks with a carbon number (CN) greater than 42 are more concentrated in the solid fractions; the groups of peaks with a CN less than 42 are more concentrated in the liquid fractions. This reflects the tendency for the higher molecular weight triglycerides to solidify and for the lower molecular weight triglycerides to stay in the liquid fractions. It is also interesting to note the differences that exist within the groups of peaks with the same CN. Although complete identification of all peaks separated on the DB-5 capillary column was not possible, it was determined for the C54 group of peaks that the first peak included the tri-unsaturated C54 triglycerides and the last peak was the trisaturated, C54 (tristearin) species. The chromatograms (Fig. 10) indicate that the last peak in each group (C44 to C54) having the same CN increased substantially in the solid fractions; the same peak almost disappeared on the chromatograms obtained with liquid fractions. This reflected the tendency for the trisaturated species of each group to concentrate in the solid fractions.

3.3 Physical Characteristics

The melting and solidification behaviour of an oil or fat as measured (DSC) by the ratio of liquid/solid at different temperatures are of great importance in assessing its use in a particular food application. The percentages of liquid oil in the various fractions as a function of temperature are presented in Tables 13 and 14.

The results of the statistical analyses (with the exception of the liquid fractions at the upper temperatures) showed significant differences

Table 13. Liquid Oil Content of Winter Butterfat and Butterfat Fractions.

Temperature (°C)	% Liquid oil in designated fraction[1,2]								
	S-29	S-26	S-23	S-19	Whole winter butterfat	L-29	L-26	L-23	L-19
0.0	9.0^{h}	10.7^{g}	12.1^{f}	12.5^{f}	15.6^{e}	23.3^{d}	24.6^{c}	26.9^{b}	28.1^{a}
5.0	15.2^{i}	17.5^{h}	19.3^{g}	19.7^{f}	24.8^{e}	32.3^{d}	34.2^{c}	37.6^{b}	39.3^{a}
10.0	23.8^{i}	27.7^{h}	29.9^{g}	30.5^{f}	39.3^{e}	47.1^{d}	49.3^{c}	53.9^{b}	57.8^{a}
15.0	31.4^{i}	36.2^{h}	39.3^{g}	40.3^{f}	51.9^{e}	67.0^{d}	71.0^{c}	76.8^{b}	79.3^{a}
20.0	36.6^{i}	44.2^{h}	48.3^{g}	49.0^{f}	64.6^{e}	80.7^{d}	85.3^{c}	93.6^{b}	95.4^{a}
25.0	37.1^{h}	47.4^{g}	54.8^{f}	57.3^{e}	75.8^{d}	89.3^{c}	94.7^{b}	98.2^{a}	98.3^{a}
30.0	46.3^{h}	58.9^{g}	67.4^{f}	70.9^{e}	87.9^{d}	97.7^{c}	98.4^{b}	99.5^{a}	99.5^{a}
35.0	61.6^{i}	74.6^{h}	83.7^{g}	87.2^{f}	97.3^{e}	98.7^{d}	99.1^{c}	100.0^{a}	99.5^{b}
40.0	83.6^{h}	92.5^{g}	96.0^{f}	97.2^{d,e}	98.1^{d}	98.8^{c}	99.4^{b}	100.0^{a}	99.6^{b}
	Melting point of designated fraction (°C)[2,3]								
	43.5^{a}	41.5^{b}	39.0^{c}	38.2^{d}	34.6^{e}	29.7^{f}	27.0^{g}	22.9^{h}	20.6^{i}

[1] The % liquid oil at various temperatures was determined by Differential Scanning Calorimetry (DSC).

[2] See Table 8, footnote 1.

[3] Melting points were determined by AOCS Method Cc 1-25.

Table 14. Liquid Oil Content of Summer Butterfat and Butterfat Fractions

Temperature (°C)	% Liquid oil in designated fraction [1,2]				
	S-29	S-19	Whole summer butterfat	L-29	L-19
0.0	9.1[e]	14.2[d]	18.2[c]	24.7[b]	29.3[a]
5.0	15.3[e]	22.2[d]	28.9[c]	34.3[b]	40.9[a]
10.0	23.5[e]	33.0[d]	42.8[c]	47.6[b]	56.7[a]
15.0	30.0[e]	43.0[d]	56.6[c]	67.1[b]	78.8[a]
20.0	32.1[e]	49.0[d]	67.4[c]	80.0[b]	96.1[a]
25.0	32.6[e]	56.9[d]	78.6[c]	89.1[b]	99.0[a]
30.0	42.2[e]	70.1[d]	90.2[c]	97.4[b]	100.0[a]
35.0	57.0[d]	85.7[c]	98.0[b]	99.6[a]	100.0[a]
40.0	78.7[b]	96.8[a]	99.9[a]	99.9[a]	100.0[a]
	Melting point of designated fraction (°C)[2,3]				
	44.4[a]	38.4[b]	33.4[c]	28.8[d]	19.7[e]

[1] See Table 13.

[2] See Table 8, footnote 1.

[3] See Table 13.

($p<0.05$) in the melting behaviour between all fractions obtained in each experiment (Tables 13 and 14). It should be noted that the differences in melting behaviour between fractions obtained from the winter butterfat are more pronounced than the differences in fatty acid composition (Tables 8 and 9). The melting behaviour of the S- and L-23 fractions as compared with the S- and L-19 fractions (winter butterfat) were significantly different ($p<0.05$); the differences in fatty acid composition of the same fractions were not significantly different. The same was true for the L-26 compared with L-29 fractions obtained from the winter butterfat. This would suggest that the arrangement of the fatty acids in the triglycerides has a more marked influence on the physical properties of butterfat fractions than does the fatty acid composition.

Figure 11 shows typical DSC crystallization curves for winter butterfat and the S-29 and L-19 fractions obtained from this fat. Figure 12 shows a comparison of the corresponding melting curves.

The crystallization and melting behaviour of the S-29 fraction differed markedly from that of the whole butterfat and the L-19 fraction. The crystallization curves obtained with the S-29 fraction showed a large high temperature peak (at ca. 20°C) which was present only as a small shoulder preceeding the low temperature peak in the whole butterfat curve; this was absent in the curve obtained with the L-19 fraction (Fig. 11). Similarly, the melting curve of the S-29 sample had a large peak at 41°C; this was almost absent in the curve obtained with the whole butterfat and did not appear in the L-19 curve. The L-19 melting curve had a large low temperature peak at 17°C, and a portion of the curve

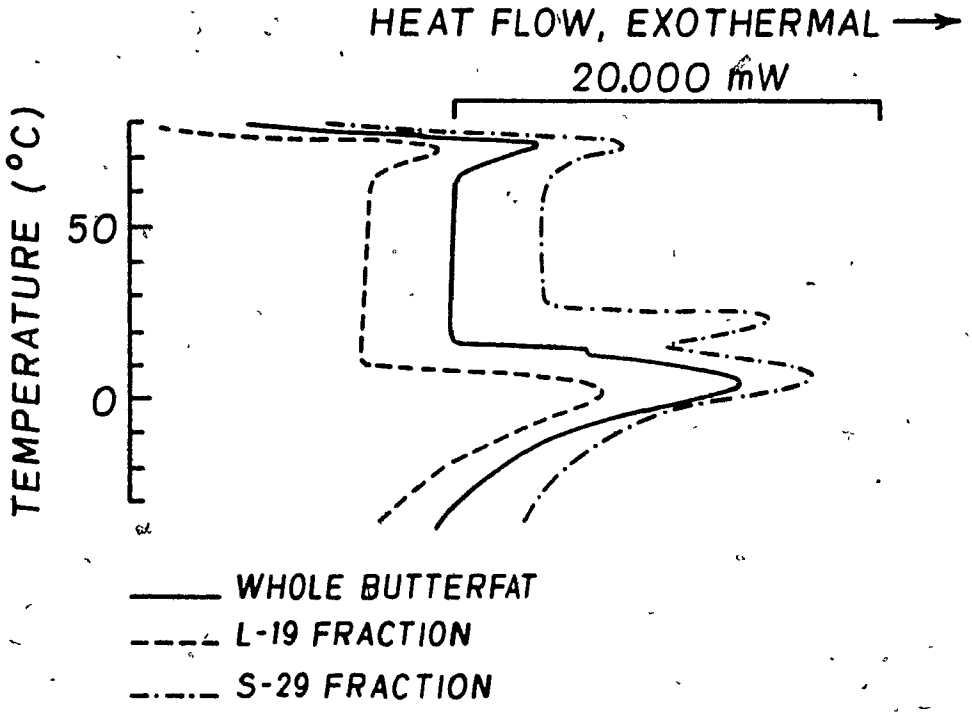


Fig. 11. Typical DSC crystallization diagrams of whole butterfat and butterfat fractions.

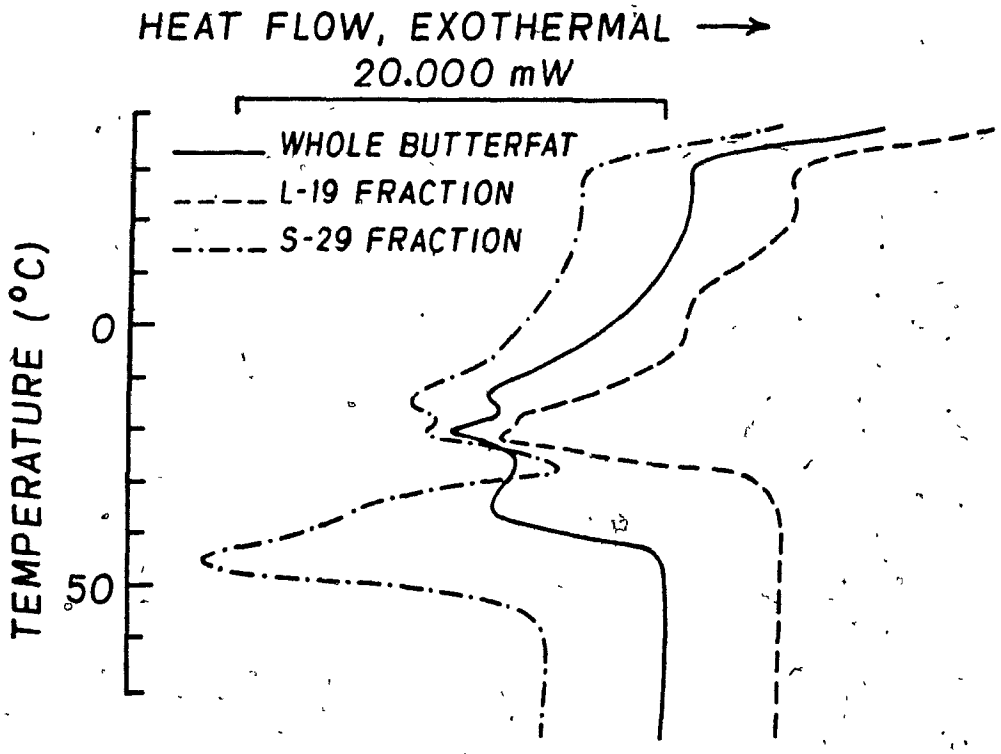


Fig. 12. Typical DSC melting diagrams of whole butterfat and butterfat fractions.

indicated there was some melting between -20°C and -5°C. This region of melting was almost absent from the other two curves (Fig. 12).

The present study has shown that butterfat can be fractionated by controlled cooling of melted butterfat, to yield products which differ markedly in their physical and chemical characteristics. These fractions might be incorporated into foods where the melting and crystallization behaviour of whole butterfat is not suitable but where the flavour of butterfat is desirable. For example, Tolboe (1984) used successfully a high-melting fraction of butterfat, which had a melting curve similar to that of the S-29 fractions obtained in the present study, in Danish pastry. The corresponding low-melting fraction was suitable as a cookie fat (Tolboe, 1984).

CHAPTER IV

EFFECTS OF THERMAL OXIDATION ON THE CONSTITUTION OF BUTTERFAT, BUTTERFAT FRACTIONS AND CERTAIN VEGETABLE OILS. 1. POLYMERIC COMPONENTS

1. INTRODUCTION

Previous work (Perkins, 1976) has shown that the thermal oxidation of a fat (heating at 180°C - 200°C in the presence of air) results in a variety of complex chemical changes in the fat, both oxidative and thermolytic. This leads to the formation of numerous volatile and nonvolatile degradation products, many of which are important from the standpoints of flavour, odour and nutrition.

Of particular interest are the nonvolatile degradation products which accumulate in the thermally oxidized fats and oils and subsequently are ingested with the food. A relationship between the nonvolatile, non-urea-adduct-forming fraction of heated vegetable oils and various toxic responses has been suggested by the early work of Crampton et al. (1953, 1956). The work of subsequent researchers (Michael et al., 1966; Artman, 1969; Artman and Smith, 1972; Ohfuji and Kaneda, 1973) supports these findings and suggests that the nonadductable monomers and oxidative dimers are the main source of toxicity.

Numerous investigations have dealt with the characterization of various components of thermally oxidized vegetable fats and oils; corresponding data for butterfat, however, is limited. There is some evidence in the early literature that butterfat may have possible nutritional

advantages over vegetable oils as a cooking fat (Johnson et al., 1956; Bhalerao et al., 1959; Coombs et al., 1965). Knowledge of the differences in composition of thermally oxidized butterfat and of vegetable oils may suggest possible factor(s) which could be responsible for the observed nutritional advantages of butterfat. Such information is also desirable in the view of current interest within the dairy industry in the fractionation of butterfat to yield products which might have specific uses in food formulation and processing (Chapter III). To the authors' knowledge, there is no information in the literature on the thermal oxidative behaviour of such butterfat fractions.

The present study deals with the characterization of nonvolatile degradation products which are formed during thermal oxidation of butterfat and butterfat fractions as well as certain vegetable oils. In particular, this study deals with the higher molecular weight compounds that form as a result of thermal oxidation of fats and oils.

2. EXPERIMENTAL

2.1 Fat and Oil Samples

Samples of winter (January) and summer (September) butterfat were prepared from fresh butter (Cooperative Agricole de la Cote Sud, Quebec) by melting the butter at 60°C, removing the top oil layer, filtering the oil through glass wool and drying the resulting product over anhydrous sodium sulfate. The oil was then refiltered (vacuum, Whatman 41 paper), flushed with nitrogen and stored at -20°C. The anhydrous butterfat was fractionated at 29 and 19°C by crystallization from molten fat to yield

solid (S) and liquid (L) fractions at each temperature. The fractionation procedure has been described previously (Chapter III).

Sunflowerseed oil (Safflo, CSP Foods Ltd., Saskatchewan) and corn oil (Mazola, Best Foods, Montreal) were purchased from a local supermarket. Canola oil and soybean oil were obtained from Canada Packers Ltd. (Montreal). None of the vegetable oils contained any preservatives.

2.2 Thermal Oxidation Procedure

A sample (100 g) of fat or oil was placed in a three-neck round-bottom flask (500 ml) and then heated (heating mantle) at 185°C ± 2°C for 8 or 16 h. A constant flow (30 ml/min, double stage regulator and flowmeter) of extra dry air (Linde, St. Laurent, Quebec) was passed into the oil during the heating period. The same cylinder of air was used throughout the study. The samples were stored at -20°C under an atmosphere of nitrogen until they were analyzed.

2.3 Gel Permeation Chromatography (GPC)

The treated and untreated samples were analyzed using a High Performance Liquid Chromatograph (HPLC) supplied by Waters Associates (Milford, Massachusetts); the instrument consisted of a Model 510 pump, a U6K Universal Injector, an R401 Differential Refractometer (attenuation 8x8) and a series of three columns (7.8 mm ID X 30 cm length; 10^3 Å, 500 Å, and 100 Å Ultrastyragel) operated at room temperature. The column packing material was highly crosslinked styrenedivinylbenzene copolymer

(<10 microns). The total permeation volume was 36 ml (or 12 ml/column), and the total void volume was 18 ml. Tetrahydrofuran (THF) stabilized with 250 ppm BHT (Anachemia, Lachine, Quebec), was used as the solvent at a flow rate of 1.0 ml/min. Peak integration was performed using a Spectra-Physics (San Jose, California) SP-4270 Integrator.

2.4 Preparation of Samples for GPC

For the analysis of intact triglycerides, the samples (30 to 50 mg) were dissolved in 1 ml of THF.

Methyl esters of the unheated and heated fats and oils were prepared by IUPAC Method 2.301 (4.2) for acid oils and fats (IUPAC, 1979) with minor modifications. The methyl esters were extracted with hexane and the resultant solution dried over anyhdrous sodium sulfate. The hexane was evaporated using a stream of nitrogen, and the methyl esters were redissolved in THF (30 to 50 mg methyl esters per 1 ml of solvent). High purity dimer and trimer acids were obtained from Emery Industries (Cincinnati, Ohio); they were converted to methyl esters by the procedure given previously. Standard mixtures of triglycerides, diglycerides, monoglycerides and free fatty acids were purchased from Sigma Chemical Co. (St. Louis, Missouri).

2.5 Fatty Acid Analysis

The preparation of methyl esters and the determination of fatty acid composition by capillary column-gas liquid chromatography was described in Chapter III.

3. RESULTS AND DISCUSSION

Figure 13 shows typical gel permeation chromatograms of butterfat and vegetable oil samples which were heated at 185°C for 16 h; chromatograms from both the intact samples and their methyl esters are shown. The dimeric and higher molecular weight peaks which increase as a result of polymerization reactions during thermal oxidation give an indication of oil deterioration.

It should be recalled that GPC separations are based strictly on differences in molecular size. Thus, the chromatographic peaks may include both nonpolar and polar components. The relative concentrations of non-polar and polar components will depend on the presence of polar functional groups and, in the case of polymerized fatty acids or triglycerides, on the type of linkage between fatty acid or triglyceride units (e.g., carbon to carbon, ether, hydroperoxide or epoxide linkage) (Ottaviani et al., 1979). It should also be noted that GPC of the intact samples gives an indication of the degree of intermolecular polymerization (i.e., reactions between fatty acids on two different triglyceride molecules) and not intramolecular polymerization (i.e., reactions between fatty acids on the same molecule). Thus, the heated oils were transformed to methyl esters to

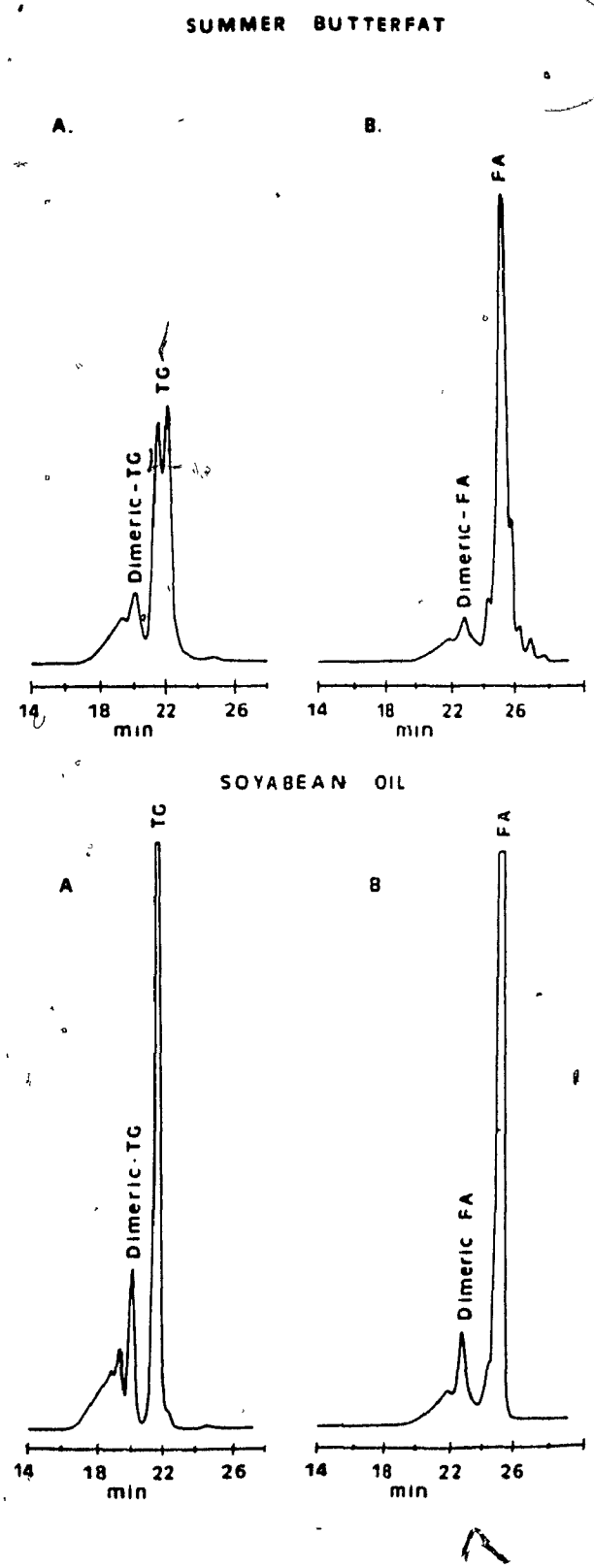


Fig. 13. Gel permeation chromatograms of thermally oxidized (185°C; 16 h) summer butterfat and soybean oil (A, intact oil; B, methyl esters).

obtain additional data on the total amounts of inter- and intramolecular fatty acid dimers and higher oligomers which were formed during the thermal oxidation treatment.

Components are eluted from gel permeation columns in the order of decreasing molecular size. The series of columns which was used in this investigation resulted in the separation of normal weight butterfat triglycerides to give two peaks; these represent two broad groups of triglycerides which differed in molecular size (Fig. 13). The second peak represented the low molecular weight triglycerides; they are eluted with diglycerides as indicated by the retention time of standard diglycerides. The vegetable oils, on the other hand, gave a single triglyceride peak. This indicates the narrower range of composition of vegetable oil triglycerides compared to butterfat triglycerides which contain from C22 to C54 (total acyl carbon number) species (Chapter III).

The results reported in Table 15 show that all of the unheated vegetable oils contained dimeric triglycerides ranging in amounts from 0.21% to 0.59%; the unheated butterfats did not contain any detected polymeric triglycerides. The occurrence of high molecular weight compounds in fresh vegetable oils is most likely the result of processes that were used in their refinement (i.e., degumming, alkali refining, bleaching, deodorization).

Table 15. Monomeric, Dimeric and Higher Oligomeric Triglycerides Constitution of Unheated and Thermally Oxidized Butterfats and Vegetable Oils.

Heating period (h)	GPC fraction	% of intact sample (peak area/total area of all peaks) Canola oil[a]	Sunflowerseed oil[a]	Corn oil[a]	Soybean oil[a]	Winter butterfat[b]	Summer butterfat[a]
0	Trimeric and higher oligomeric	nd[c]	nd	nd	nd	nd	nd
	Dimeric	0.21	0.59	0.21	0.25	nd	nd
	Monomeric (TG)	97.06	97.06	95.83	98.10	50.01	52.26
	Monomeric (TG, DG)	2.45	2.04	3.51	1.36	49.54	47.24
	Monomeric (FFA)	0.29	0.31	0.45	0.30	0.45	0.50
8	Trimeric and higher oligomeric	7.70	10.33	2.86	8.82	4.93	5.51
	Dimeric	12.17	15.85	8.90	13.51	9.74	9.34
	Monomeric (TG)	77.58	72.14	84.45	77.43	39.63	41.63
	Monomeric (TG, DG)	2.40	1.44	3.44	tr[d]	45.70	43.23
	Monomeric (FFA)	0.16	0.23	0.35	0.24	tr	0.28
16	Trimeric and higher oligomeric	19.65	23.10	9.60	21.14	9.12	11.48
	Dimeric	14.67	17.49	13.76	15.05	12.81	13.16
	Monomeric (TG)	62.99	59.23	72.90	63.55	34.23	34.27
	Monomeric (TG, DG)	2.69	tr	3.50	tr	43.84	41.03
	Monomeric (FFA)	tr	0.18	0.23	0.27	tr	0.05

[a] Each value for 8- and 16-h heating represents the mean of 3 replicate samples, analyzed in duplicate.
[b] Each value represents the mean of one sample, analyzed in duplicate.
[c] Not detected.
[d] Trace (not integrated).

The sunflowerseed, soybean and canola oils, after both 8 and 16 h of thermal oxidation, contained substantially higher amounts of both dimeric and higher oligomeric triglycerides than did either the corn oil or butterfat samples (Table 15). It should be noted that with the corn oil samples, after 16 h of thermal oxidation a very viscous and dark coloured material which was not soluble in chloroform, ethyl ether or acetone remained on the inner walls of the flask after the sample was removed; this was not observed with any of the other samples. This material could contain high molecular weight components which were not accounted for in the GPC results from the corn oil samples which were heated for 16 h. The results obtained with all of the fat and oil samples indicate that the rates of dimeric triglyceride formation, during the first 8 h of heating, exceeded the rates of trimeric and higher oligomeric triglyceride formation. The fastest rates of formation of all polymers occurred in the sunflowerseed, soybean and canola oils. The rate of dimer formation in the sunflowerseed, soybean and canola oils decreased during the second 8 h of heating, while the amounts of higher oligomeric triglycerides continued to increase at a steady rate throughout the 16 h heating period. The rates of formation of dimers as compared with the higher oligomers in the sunflowerseed, soybean and canola oils suggest that dimers are formed preferentially and that the higher oligomers were formed from the dimers (addition polymerization).

The corn oil behaved more like the butterfat samples with respect to intermolecular polymerization than did the other three vegetable oils. This is somewhat surprising in view of the considerable differences in their fatty acid compositions (Table 16). It seems reasonable to assume

Table 16. Fatty Acid Composition of Winter and Summer Butterfat and Selected Vegetable Oils.

Fatty acid group	Methyl esters (Wt %)[a]					
	Canola oil	Sunflowerseed oil	Corn oil	Soybean oil	Winter butterfat	Summer butterfat
Saturated	7.43	10.74	12.99	15.07	70.48	66.73
Monounsaturated	58.16	18.00	26.83	22.66	24.57	27.36
Polyunsaturated	34.17	70.90	60.17	61.86	1.90	2.36
Other	0.24	0.35	-	0.42	3.04	3.55

[a] Each sample was analyzed in duplicate.

that the probability of intermolecular polymerization increases as the degree of unsaturation of an oil increases. The results reported here, however, suggest that (at least during the first 8 h of heating) there are factors other than degree of unsaturation which have a marked influence on intermolecular polymerization. One such factor could be the presence of naturally occurring antioxidants. It is known that untreated corn oil contains a certain amount of tocopherol (Sonntag, 1979) which could explain its slower rate of polymerization during 8 h of thermal oxidation compared to the other vegetable oils.

Tables 17 and 18 show that there are some differences in the intermolecular polymerization of the butterfat fractions in comparison to whole butterfat. The chemical and physical properties of the whole butterfat and butterfat fractions which were used in the present study were described in Chapter III.

The liquid fraction (L-19; Table 17) obtained from the winter butterfat showed some stability toward thermal oxidation (8-hour heat treatment) as compared to the other samples; this sample, however, had the highest degree of unsaturation (Chapter III). After 16 h of heating, the solid fraction (S-29; Table 17) exhibited the greatest resistance to intermolecular polymerization. With the summer butterfat samples (Table 18), the S-29 fraction contained the lowest level of total polymeric triglycerides after both 8 and 16 h of thermal oxidation; the L-19 fraction contained the highest levels of polymeric triglycerides. A comparison of the results obtained with winter and summer butterfats shows that the levels of dimeric triglycerides in the samples heated for 8 and 16 h were very similar. The main differences were in the levels of high molecular

Table 17. Monomeric, Dimeric and Higher Oligomeric Triglycerides Constitution of Unheated and Thermally Oxidized Winter Butterfat and Butterfat Fractions.

Heating period (h)	GPC fraction	% of intact sample (peak area/total area of all peaks)[a] Fraction[b] S-29	Fraction S-19	Whole butterfat	Fraction L-29	Fraction L-19
0	Trimeric and higher oligomeric	nd[c]	nd	nd	nd	nd
	Dimeric	nd	nd	nd	nd	nd
	Monomeric (TG)	65.86	56.05	50.01	44.83	40.55
	Monomeric (TG, DG)	33.74	43.54	49.54	54.55	58.82
	Monomeric (FFA)	0.39	0.41	0.45	0.62	0.64
8	Trimeric and higher oligomeric	4.38	4.62	4.93	5.24	5.00
	Dimeric	8.86	9.36	9.74	8.45	7.82
	Monomeric (TG)	55.38	45.93	39.63	35.16	30.73
	Monomeric (TG, DG)	31.30	40.10	45.70	50.86	56.25
	Monomeric (FFA)	0.11	tr[d]	tr	0.29	0.20
16	Trimeric and higher oligomeric	7.95	9.04	9.12	13.33	12.22
	Dimeric	12.48	12.24	12.81	11.74	11.71
	Monomeric (TG)	50.39	40.42	34.23	27.14	22.76
	Monomeric (TG, DG)	29.18	38.29	43.84	47.78	53.32
	Monomeric (FFA)	tr	tr	tr	tr	tr

[a] Each value represents the mean of one sample, analyzed in duplicate.
[b] Fraction designation indicates physical state (solid, liquid) and fractionation temperature (°C).
[c] Not detected.
[d] Trace (not integrated).

Table 18. Monomeric, Dimeric and Higher Oligomeric Triglycerides Constitution of Unheated and Thermally Oxidized Summer Butterfat and Butterfat Fractions.

Heating period (h)	GPC Fraction	% of intact sample (peak area/total area of all peaks)[a] Fraction[b] S-29	Fraction S-19	Whole butterfat	Fraction L-29	Fraction L-19
0	Trimeric and higher oligomeric	nd[c]	nd	nd	nd	nd
	Dimeric	nd	nd	nd	nd	nd
	Monomeric (TG)	68.69	59.22	52.26	49.38	45.12
	Monomeric (TG, DG)	31.08	40.45	47.24	50.22	54.40
	Monomeric (FFA)	0.23	0.30	0.50	0.40	0.48
8	Trimeric and higher oligomeric	4.75	5.48	5.51	6.33	6.62
	Dimeric	8.83	9.40	9.34	8.48	8.51
	Monomeric (TG)	59.46	48.34	41.63	39.01	34.52
	Monomeric (TG, DG)	26.84	36.57	43.23	46.01	50.08
	Monomeric (FFA)	0.12	0.21	0.28	0.18	0.27
16	Trimeric and higher oligomeric	9.90	10.77	11.48	13.75	15.34
	Dimeric	12.48	12.99	13.16	11.75	11.39
	Monomeric (TG)	52.15	40.90	34.27	31.54	26.71
	Monomeric (TG, DG)	25.26	35.09	41.03	42.83	46.32
	Monomeric (FFA)	0.20	0.26	0.05	0.13	0.23

[a] Each value represents the mean of 3 replicate samples, analyzed in duplicate.
[b] Fraction designation indicates physical state (solid, liquid) and fractionation temperature (°C).
[c] Not detected.

weight polymers with the summer butterfat and fractions containing larger amounts of trimeric and higher oligomeric triglycerides than the corresponding winter samples. This reflected the higher proportion of unsaturated fatty acids in the summer butterfat as compared to the winter butterfat (Table 16) (Chapter III).

Table 19 summarizes the GPC data from heated fat and oil samples (16 h) which were transformed to the corresponding methyl esters prior to GPC. These results give an indication of the total amount of polymerization (both inter- and intramolecular) which has occurred. It is reasonable to expect that the probability of having more than one unsaturated fatty acid in a triglyceride molecule will be greater with samples having high levels of unsaturated fatty acids. This, in turn, should result in an increased probability of intramolecular polymerization (Pokorny et al., 1976b). It was observed that the sunflowerseed oil had undergone the highest degree of both inter- and intramolecular polymerization among all the fats and oils that were studied. On the other hand, the corn oil sample was relatively resistant to polymerization compared to the other vegetable oils. It is interesting to note that, despite the fatty acid composition of the corn oil, the heated oil contained primarily intermolecular polymers as indicated by the relatively low value for trimeric fatty acid methyl esters (Table 19). Trimeric fatty acids can arise only if intramolecular polymerization reactions have occurred. As indicated previously, the results for corn oil (16-h sample) may be lower than the actual values due to losses of polymeric material on the inner walls of the oxidation flask. The gel permeation chromatograms from the fatty acid methyl esters showed that none of the fats and oils studied

Table 19. Monomeric, Dimeric and Higher Oligomeric Fatty Acid Constitution of Thermally Oxidized (185°C, 16 h) Butterfats, Butterfat Fractions and Selected Vegetable Oils.

GPC fraction	Fatty acid methyl esters, % (peak area/total area)			
	Canola oil[a]	Sunflowerseed oil[a]	Corn oil[a]	Soybean oil[a]
Trimeric and higher oligomeric	5.98	5.64	2.96	6.47
Dimeric	11.04	14.89	9.24	12.50
Monomeric (fatty acids)	82.98	79.47	87.79	81.03

	Fractions from winter butterfat				
	S-29[b]	S-19[b]	Whole butterfat[b]	L-29[b]	L-19[b]
Trimeric and higher oligomeric	3.97	4.31	4.21	8.18	7.07
Dimeric	9.24	8.94	9.18	7.20	7.82
Monomeric (fatty acids)	86.79	86.75	86.61	84.62	85.11

	Fractions from summer butterfat				
	S-29[a]	S-19[a]	Whole butterfat[a]	L-29[a]	L-19[a]
Trimeric and higher oligomeric	3.59	4.50	4.96	6.44	7.92
Dimeric	7.70	8.63	8.97	8.51	8.06
Monomeric (fatty acids)	88.71	86.88	86.07	85.03	84.02

[a] Each value represents the mean of 3 replicate samples, analyzed in duplicate.
[b] Each value represents the mean of one sample, analyzed in duplicate.

contained polymeric fatty acids with more than three component fatty acids.

The results obtained with the butterfat samples (both winter and summer) indicate that, in general, the total amount of polymerization increased as the level of unsaturated fatty acids in the unheated fats increased (Table 19). Pokorny et al. (1976b) stated that it is mainly the polyenoic fatty acids which participate in polymerization reactions of lipids. The present study indicates that this is not necessarily true, because the levels of polyenoic fatty acids in the butterfat samples alone do not account for the observed levels of polymerization. It would appear that, under conditions of thermal oxidation, both monoenoic and polyenoic fatty acids, and perhaps saturated fatty acids, once oxidized, have the potential to participate in polymerization reactions.

The results reported in the present chapter indicate that butterfat and the fractions of butterfat are much more stable to thermal oxidation than are certain vegetable oils (canola, sunflowerseed and soybean). The corn oil also exhibited a high degree of stability after 8 h of heating. However, the 16-h corn oil data was less certain due to the presence of an insoluble material which could not be removed from the oxidation flask; this was believed to contain highly polymerized oil and was not observed with any of the other samples. The data also suggests that the degree of unsaturation of a fat or oil alone does not control the extent or rate of polymerization reactions during thermal oxidation. It is certain that unsaturation is involved in polymerization of fats; other factors, however, also could be important as would be indicated from the results with corn oil (8-h heat treatment) and with the L-19 fraction from winter butterfat (8-h heat treatment). It should be pointed out that, although the

formation of polymeric compounds in heated fats and oils is considered to be an indication of the extent of degradation, the monomeric triglycerides or fatty acids may also be degraded by thermal or oxidative changes.

CHAPTER V

EFFECTS OF THERMAL OXIDATION ON THE CONSTITUTION OF BUTTERFAT, BUTTERFAT FRACTIONS AND CERTAIN VEGETABLE OILS.

II. POLAR AND NONPOLAR COMPONENTS

1. INTRODUCTION

It is known that the thermal oxidation of a fat results in a variety of oxidative and thermolytic reactions in the fat which lead to the formation of numerous volatile and nonvolatile degradation products. Many investigations have dealt with the assessment of the thermal oxidative behaviour of vegetable fats and oils; these fats and oils are used in considerable quantities for deep fat frying of foods and thus a knowledge of their decomposition pathways and products is of great practical and nutritional importance.

Butter is also used in food preparation, both as a component and as a frying medium; this is primarily because of its favourable flavour characteristics. There is, however, little published information on the thermal oxidative behaviour of butterfat and the composition of the resultant nonvolatile degradation products. Butterfat is unique among all of the naturally occurring fats and oils in its chemical and physical characteristics. More than 400 different fatty acids have been found to be present in butterfat (Patton and Jensen, 1975). A large proportion of the fat is comprised of saturated fatty acids (ca. 66-70%) of which approximately 12-13% is short chain fatty acids (C4:0 to C10:0); the main unsaturated fatty acid in butterfat is oleic acid (ca. 19-21%) (Chapter III). Vegetable oils, such as corn oil and soybean oil, on the other hand,

contain primarily long chain unsaturated fatty acids; corn oil and soybean oil contain 87% and 84% of total unsaturated fatty acids, respectively (Chapter IV). Amer and Myer (1974) found that when modified butter (which contained 30% sunflower oil) was heated (90°C for 20, 40 and 60 h), the rate of oxidation was much more rapid in the modified butter than in normal butter. Thus major differences would be expected in the composition of decomposition products from heated butterfat compared to those of heated vegetable oils.

The present study deals with the characterization of nonvolatile degradation products which are formed during the thermal oxidation of butterfat and butterfat fractions as well as certain vegetable oils. The gel permeation properties of the heated fats and oils (both at the triglyceride and fatty acid levels) have been reported in Chapter IV. The present chapter deals with the polar and nonpolar products that form as a result of thermal oxidation of fats and oils.

2. EXPERIMENTAL

2.1 Fat and Oil Samples

Anhydrous butterfat was prepared from fresh summer (September) butter (Cooperative Agricole de la Cote Sud, Quebec). The anhydrous butterfat was fractionated at 29 and 19°C by crystallization from molten fat to yield solid (S) and liquid (L) fractions at each temperature. The preparation of anhydrous butterfat, the fractionation procedure, and the

chemical and physical characteristics of the fractions have been described previously (Chapter III).

Sunflowerseed oil (Safflo, CSP Foods Ltd., Saskatchewan) and corn oil (Mazola, Best Foods, Montreal) were purchased from a local supermarket. Canola oil and soybean oil were obtained from Canada Packers Ltd, (Montreal). None of the vegetable oils contained any preservatives.

2.2 Thermal Oxidation Procedure

A continuous heat treatment was used by which 100 g samples of anhydrous fat or oil were placed in round-bottom flasks and maintained at 185 ± 2°C for 8 or 16 h in the presence of air (30 ml/min); the method has been described previously (Chapter IV).

2.3 Chromatographic Separation of Polar and Nonpolar Components

The thermally oxidized oils were separated into polar and nonpolar fractions by column chromatography on silica gel 60 (Merck No. 7734). The method of Guhr and Waibel (as reported by Waltking and Wessels, 1981) was used, with minor modifications. The preparation of the silica gel and the column were as described by Waltking and Wessels (1981). A measured quantity (1.0 g) of thermally oxidized oil was dissolved in 10 ml of the first eluting solvent mixture (petroleum ether - ethyl ether, 87:13) and added to the top of the column. An additional 10 ml of the same solvent mixture was used to rinse the beaker, funnel, and inner walls of the

column. The nonpolar fraction was eluted with 150 ml of the petroleum ether-ethyl ether mixture into a round-bottom flask (250 ml). The polar components were eluted into a second round-bottom flask with 50 ml of the petroleum ether-ethyl ether mixture followed by 100 ml of isopropyl alcohol. The solvents were evaporated using a rotary evaporator and the weights of the fractions were determined. The quality of the separations were checked by thin layer chromatography (Waltking and Wessels, 1981). The modification in the elution of the polar components was made following preliminary analyses which indicated that the use of 150 ml of the first solvent mixture (petroleum ether - ethyl ether, 87:13) to elute the polar fraction (Waltking and Wessels, 1981) resulted in incomplete recoveries of polar components from the columns.

2.4 Gel Permeation Chromatography (GPC)

The nonpolar and polar fractions which were separated from the thermally oxidized oils were analyzed (as intact samples) by GPC using high pressure liquid chromatography (Chapter IV).

2.5 Fatty Acid Analysis

The fatty acid compositions of the untreated samples and the nonpolar fractions from the treated samples were determined by capillary column-gas chromatography of the methyl esters (Chapter III).

3. RESULTS AND DISCUSSION

The amounts of total polar components in the nonvolatile fractions of the thermally oxidized vegetable oil and butterfat samples are reported in Tables 20 and 21, respectively. According to the method which was used in this study to separate the heated oils into nonpolar (NP) and polar (P) fractions, the NP fractions contained primarily unaltered triglycerides and a small amount of unsaponifiable matter; the P fractions contained some components which occur in unheated fats and oils, such as monoglycerides, diglycerides and free fatty acids, as well as polar transformation products which were formed during thermal oxidation (Waltking and Wessels, 1981). The size of the polar fraction gives an indication of the degree of fat deterioration (Billek et al., 1978). Also reported in Tables 20 and 21, are the distributions of polymeric material in the P fractions as determined by gel permeation chromatography. Figure 14 shows typical gel permeation chromatograms of P and NP fractions from butterfat and vegetable oil samples which were heated at 185°C for 16 h.

Under the conditions of heat treatment that were used, the thermally oxidized sunflowerseed, soybean and canola oils contained substantially higher amounts of total polar components (after both 8 and 16 h) than did either the butterfat samples or the corn oil (Tables 20 and 21). Similar data for butterfat have not been reported previously. Direct comparisons of the vegetable oil results with those reported in the literature (Gere, 1982; Yoshida and Alexander, 1982) also cannot be made due to differences in heat treatments that were used by the various investigators. As a reference point, Billek (1979) has suggested, for frying oils, a level of

Table 20. Total Polar Components and the Composition of Polymeric Triglycerides in the Polar Fractions from Thermally Oxidized Vegetable Oils.

Sample	Heating time (h)	Total polar components (%)[a]	Distribution of polymeric triglycerides in polar fraction (%)[b]			
			Higher oligomers	Dimers	Monomers (including DG, MG)	Free fatty acids
Sunflowerseed oil	8	36.05 (± 0.34)	30.1	37.0	32.2	0.8
	16	52.61 (± 0.38)	40.2	31.4	28.1	0.4
Soybean oil	8	31.89 (± 0.65)	25.8	37.8	35.6	0.8
	16	47.42 (± 0.04)	41.0	30.3	28.2	0.4
Canola oil	8	31.69 (± 0.24)	24.5	33.7	41.0	0.7
	16	48.00 (± 0.52)	35.2	29.0	35.5	0.3
Corn oil	8	20.56 (± 0.99)	16.6	39.0	42.9	1.5
	16	32.61 (± 0.88)	29.1	36.2	33.9	0.8

[a] Values reported are the percentage by weight of polar components in the nonvolatile fractions of the heated fats. Heating experiments were performed in triplicate. The deviation is the average deviation from the mean of three replicate samples, analyzed in duplicate.

[b] Each value represents the mean of one sample, analyzed in duplicate.

Table 21. Total Polar Components and the Composition of Polymeric Triglycerides in the Polar Fractions from Thermally Oxidized Butterfat (BF) and Butterfat Fractions.

Sample	Heating time (h)	Total polar components (%)[a]	Distribution of polymeric triglycerides in polar fraction (%)[b]			
			Higher Oligomers	Dimers	Monomers (including DG, MG)	Free fatty acids
S-29 BF fraction[c]	8	26.92 (±0.52)	17.6	29.0	52.5	1.0
	16	38.53 (±0.38)	22.3	28.7	48.4	0.6
S-19 BF fraction	8	27.84 (±0.34)	17.8	28.7	52.5	1.0
	16	39.74 (±1.1)	22.6	28.7	48.1	0.5
Whole BF	8	28.72 (±0.58)	18.3	28.7	51.7	1.3
	16	43.08 (±0.59)	24.2	28.6	46.7	0.5
L-29 BF fraction	8	27.08 (±0.11)	24.3	27.2	47.5	1.0
	16	39.73 (±0.57)	30.4	26.2	43.0	0.4
L-19 BF fraction	8	27.12 (±0.62)	22.1	27.3	49.5	1.1
	16	40.23 (±0.04)	37.1	24.7	37.6	0.6

[a] See Table 20

[b] See Table 20

[c] Fraction designation indicates physical state (solid, liquid) and fractionation temperature (°C).

30% of total polar compounds in the oil as the point at which the oil is considered to be deteriorated and should be discarded.

The low levels of polar compounds in the corn oil compared to the other samples are somewhat surprising in view of the relatively high content of unsaturated fatty acids in this oil (Table 22); this data is consistent with the relatively low overall levels of both inter- and intramolecular fatty acid dimers and higher oligomers in the thermally oxidized corn oil which were reported in Chapter IV. It should be noted that with the corn oil samples, after 16 h of thermal oxidation, an insoluble substance (insoluble in chloroform, ethyl ether or acetone) remained on the inner walls of the flask after the sample was removed; this was not observed with any of the other samples. This substance represented approximately 2-3% of the original weight of the oil and could contain highly degraded oil which was not accounted for in the analysis of the nonvolatile fractions of the heated oil.

Figure 14 shows that the bulk of the dimeric and higher oligomeric triglycerides which were formed in the fats and oils as a result of thermal oxidation, were found in the P fractions. This indicates that these polymeric compounds contained polar functional groups and/or polar linkages between the fatty acid or triglyceride units (e.g., ether, hydroperoxide or epoxide linkage). The analysis of the NP fractions (quantitative data not shown) indicated that the 8 h - NP fractions contained from 0.3% to 0.6% dimeric triglycerides and the 16 h - NP fractions contained from 0.4% to 1.3% dimeric triglycerides. Polymeric triglycerides with three or more component triglycerides were not detected in the NP fractions from any of the thermally oxidized fats and oils. It

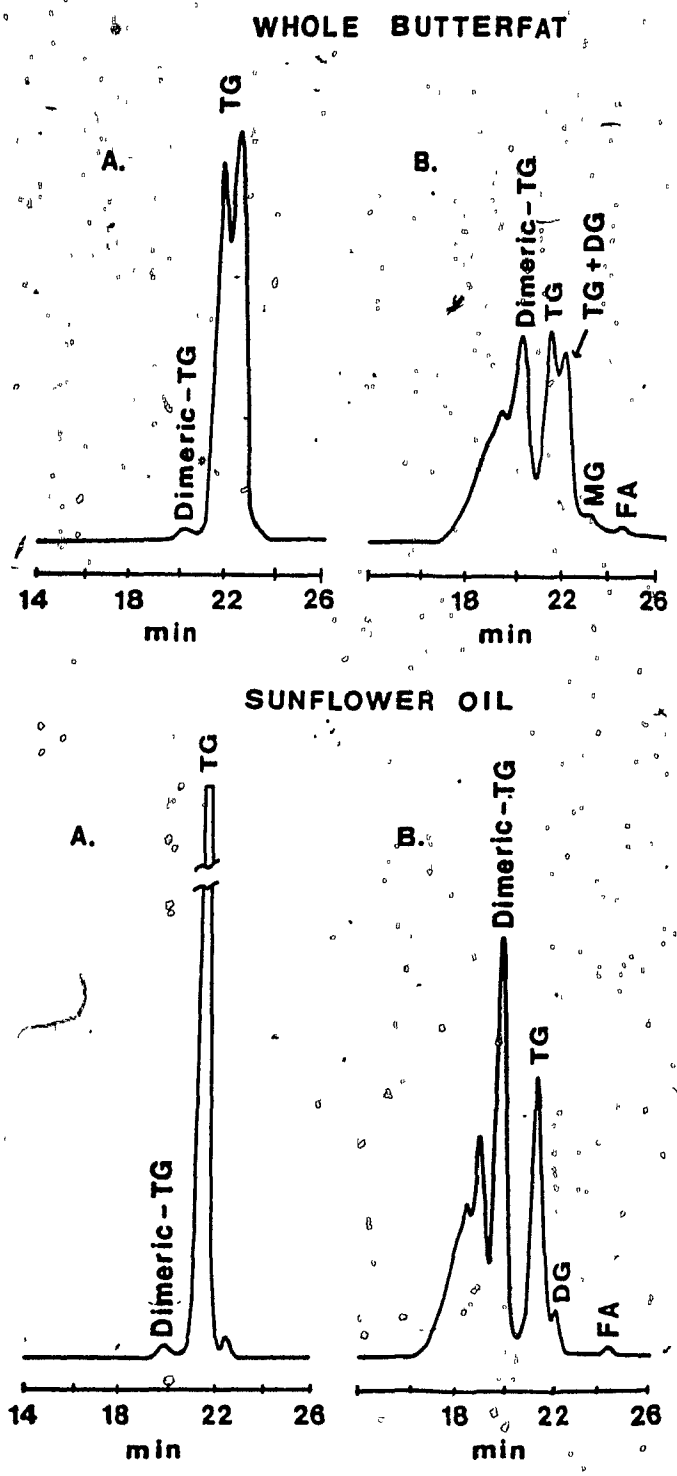


Fig. 14. Gel permeation chromatograms of nonpolar and polar fractions which were isolated from thermally oxidized (185°C; 16 h) butterfat and sunflowerseed oil (A, nonpolar fraction; B, polar fraction).

should be kept in mind that the intact samples were separated and analyzed. Thus nonpolar fatty acid polymers which might be present in the heated oils will not be detected if they occur as constituents of polar triglycerides or polymeric triglycerides.

A comparison of the compositions of polymeric material in the P fractions from the vegetable oil samples (Table 20) and the butterfat samples (Table 21) shows that the butterfat samples contained higher proportions of monomeric material (i.e., triglycerides, diglycerides, and monoglycerides) in the P fractions than did any of the vegetable oils. The higher levels of total polar components in the butterfat samples compared with the corn oil sample may have been due to a greater proportion of products of hydrolysis (i.e., diglycerides, monoglycerides, and free fatty acids) in the P fractions rather than a greater proportion of oxygenated and polymeric triglycerides. With the butterfat samples, the GPC method which was used did not permit adequate separation of diglycerides and monoglycerides from the monomeric triglycerides (Fig. 14) because of the complex triglyceride composition of butterfat. Thus the extent of hydrolytic reactions which occurred cannot be ascertained from this data. The level of free fatty acids in the P fractions also may not be a good indicator of the extent of hydrolysis, since these products may be lost with the volatile components during thermal oxidation. It seems reasonable to assume that this effect would be more pronounced with the butterfat samples since they contain a large proportion of short chain fatty acids.

Table 21 shows that there are some differences in the amounts of polar components as well as in the distributions of polymeric triglycerides in the butterfat fractions in comparison to whole butterfat. The solid butterfat fractions contained lower levels of total polar components compared to the whole butterfat; this corresponded to the lower levels of unsaturated fatty acids in the solid fractions (Table 23) (Chapter III). The liquid fractions, contained similar or slightly lower levels of total polar components compared to the whole butterfat; these samples, however, contained the highest levels of unsaturated fatty acids among all of the butterfat samples (Table 23) (Chapter III).

The GPC results shown in Table 21 indicate that as the degree of unsaturation in the butterfat samples increased, the extent of formation of polymeric triglycerides during thermal oxidation also increased. The liquid fractions contained higher levels of dimeric and higher oligomeric triglycerides in their degradation products than did the solid fractions; the solid fractions contained higher levels of oxygenated triglycerides and/or hydrolysis products in their degradation products. Thus the main reaction pathways which occur during the thermal oxidation of solid and liquid butterfat fractions are different.

The nonpolar fractions from the thermally oxidized fats and oils were further characterized according to their fatty acid compositions using capillary column-gas chromatography of the methyl esters. Tables 22 and 23 report the fatty acid compositions of the unaltered triglycerides (NP fractions) in the unheated and heated samples. As the fats and oils were heated, the levels of unsaturated fatty acids in the NP fractions progressively decreased as a result of the degradation of these acids

Table 22. Composition of Unsaturated Fatty Acids in Nonpolar Fractions from Thermally Oxidized Vegetable Oils.

Sample	Heating time (h)	Methyl esters (Wt %)[a] C18:1	C18:2	C18:3
Sunflowerseed oil	0	18.0	70.7	0.2
	8	19.4	65.8	0.2
	16	20.9	62.6	0
Soybean oil	0	22.6	53.2	8.7
	8	23.4	50.2	6.5
	16	25.8	47.7	5.2
Canola oil	0	55.7	24.4	9.7
	8	59.9	18.6	5.3
	16	65.3	16.1	3.4
Corn oil	0	26.8	59.5	0.7
	8	27.4	56.3	0.7
	16	29.0	54.8	0.5

[a] Each value for 8- and 16-h heating represents the mean of three replicate samples, analysed in duplicate.

Table 23. Composition of Unsaturated Fatty Acids in Nonpolar Fractions from Thermally Oxidized Butterfat (BF) and Butterfat Fractions.

Sample	Heating time (h)	Methyl esters (Wt %)[a] C18:1	C18:2	C18:3
S-29 BF Fraction	0	16.3	1.3	0.6
	8	13.1	0.6	0.1
	16	10.9	0.3	0
S-19 BF Fraction	0	19.2	1.5	0.7
	8	16.2	1.0	0.2
	16	13.6	0.6	0
Whole BF	0	20.5	1.6	0.8
	8	17.9	1.0	0.2
	16	15.3	0.5	0
L-29 BF Fraction	0	21.5	1.8	0.9
	8	19.0	1.2	0.2
	16	16.8	0.7	0
L-19 BF Fraction	0	22.7	1.8	0.9
	8	20.1	1.1	0.3
	16	18.4	0.6	0

[a] Each value represents the mean of three replicate samples, analysed in duplicate.

by thermal oxidative reactions. With the vegetable oil samples (Table 22), decreases in the oleic acid content of the oils are not apparent and in fact, the content of oleic acid in the NP fractions appears to increase with increased time of heating. This is because the levels of the different fatty acids in a given sample are relative to one another and the initial levels of saturated fatty acids in the vegetable oils is low. Thus decreases in linoleic and linolenic acids result in increased levels of oleic acid, even though some oleic acid may be consumed in thermal oxidative reactions. With the butterfat samples (Table 23), decreases in the levels of unsaturated fatty acids with heating resulted in corresponding increases in the levels of saturated fatty acids. It is worth noting that with the butterfat samples, regardless of the initial level of unsaturated fatty acids in the fat, the reductions in the levels of these acids (after both 8 and 16 h) were similar for all samples; absolute values of the unsaturated fatty acids in the total nonvolatile fractions will be different due to the different sizes of the NP fractions.

The results reported in the present paper lend further support to our initial observations (Chapter IV) that butterfat and the fractions of butterfat are much more stable to thermal oxidation than are certain vegetable oils (canola, sunflowerseed and soybean). The data also suggests that the degree of unsaturation of a fat or oil alone does not control the extent or rate of thermal oxidative reactions. As indicated from the results with the corn oil and the liquid butterfat fractions, factors other than the degree of unsaturation could be important in determining the extent of degradation during thermal oxidation. With the liquid butterfat

fractions, it is possible that an antioxidant factor(s) is concentrated in these fractions by the fractionation process; this could explain the thermal oxidative stability of the liquid fractions compared to the whole butterfat. Further work is being conducted in this laboratory to provide more detailed information on the constitution of the butterfat fractions and to determine the nature of the antioxidant factor(s) in the liquid butterfat fractions.

CHAPTER VI

ANALYSIS OF THERMALLY OXIDIZED BUTTERFAT, BUTTERFAT FRACTIONS AND CERTAIN VEGETABLE OILS FOR CYCLIC MONOMERS

1. INTRODUCTION

Cyclic monomers, because of their potential toxicity, are an important class of compounds which may be present in foods as a consequence of processing and cooking. They are known to arise from the intramolecular condensation of C18 polyunsaturated fatty acids (Michael, 1966a and 1966b; Gente and Guillaumin, 1977) and their occurrence has been demonstrated in vegetable oils which were used for deep fat frying (Meltzer et al., 1981; Frankel et al., 1984). Meltzer et al. (1981), after low temperature crystallization of the hydrogenated methyl esters, were able to detect cyclic fatty acids (0.3% to 0.6%) in heated (195°C for 52-104 h) soybean oil under both continuous and intermittent heating conditions. Frankel et al. (1984) analyzed vegetable fat and oil samples from food outlets in both the United States and the Middle East (Israel and Egypt) for their content of cyclic fatty acid monomers. The samples from the USA contained from 0.1% to 0.5% cyclic monomers; the samples from Israel and Egypt had values which ranged from 0.2% to 0.7%. Fresh vegetable oils, prior to heating, have also been shown (Guillaumin et al., 1977) to contain small amounts of cyclic monomers (0.1% to 0.2%); this could be the result of previous processing and refinement.

The toxicity of feeding low levels of cyclic monomers has been demonstrated in laboratory experiments with animals. Iwaoka and Perkins (1976) in experiments with rats showed that the incorporation of low levels (0.15%) of purified cyclic fatty acid methyl esters (methyl ω(2-alkyl cyclohexadienyl) carboxylic acids) in diets which contained 8% or 10% protein and 15% corn oil caused depressed growth and fatty livers due to the accumulation of lipid. Further studies by these authors (1978) showed that feeding the same levels of cyclic fatty acid methyl esters resulted in decreased lipogenesis in the livers of rats which were fed 8% and 10% protein and elevated lipogenesis in the adipose tissue of rats which were fed 10% protein diets.

The present study deals with the determination of cyclic monomers in thermally oxidized butterfat and butterfat fractions as well as in certain vegetable oils. Although butterfat is used extensively in food preparation, little is known about the composition of the nonvolatile products of thermal oxidation and in particular, the occurrence of cyclic monomers in thermally oxidized butterfat.

2. EXPERIMENTAL

2.1 Fat and Oil Samples

Anhydrous butterfat was prepared from fresh summer (September) butter (Cooperative Agricole de la Cote Sud, Quebec). The anhydrous butterfat was fractionated at 29 and 19°C by crystallization from molten

fat to yield solid (S) and liquid (L) fractions at each temperature. The preparation of anhydrous butterfat, the fractionation procedure, and the chemical and physical characteristics of the fractions have been described previously (Chapter III).

Sunflowerseed oil (Safflo, CSP Foods Ltd., Saskatchewan) and corn oil (Mazola, Best Foods, Montreal) were purchased from a local supermarket. Canola oil and soybean oil were obtained from Canada Packers Ltd. (Montreal). None of the vegetable oils contained any preservatives.

2.2 Thermal Oxidation Procedure

A continuous heat treatment was used by which 100 g samples of anhydrous fat or oil were placed in round bottom flasks and maintained at 185 $\pm$ 2°C for 8 or 16 h in the presence of air (30 ml/min); the method has been described previously (Chapter V).

2.3 Determination of Cyclic Monomers

The technique which was used for the determination of cyclic monomers was a modification of the procedure which was described by Meltzer et al. (1981); the procedure involved the following steps:

2.3.1 Preparation of methyl esters

Methyl esters of the heated fats and oils were prepared by the IUPAC Method 2.301 (4.2) for acid oils and fats (IUPAC, 1979) with minor modifications. Approximately 1 g of anhydrous lipid was heated under

reflux for 15 min with a sodium methylate solution (1 g Na per 100 ml anhydrous methanol; 10 ml). Methanolic hydrochloric acid solution (1 N; 13 ml) was then added and heating was continued for an additional 10 minutes. The flask was cooled under running water and the mixture was transferred to a separatory funnel with the aid of distilled water (25 ml). The methyl esters were extracted twice with hexane (8 ml). The hexane extracts were combined and then washed several times with distilled water. The resultant hexane solution was dried over anhydrous sodium sulfate, filtered, and the hexane was evaporated under a stream of nitrogen.

2.3.2 Micro-hydrogenation of methyl esters

The fatty acid methyl esters (50 mg) were dissolved in ethyl acetate (10 ml) and hydrogenated (platinum oxide, 50 mg) using a micro-hydrogenation unit supplied by Supelco (Bellefonte, Pennsylvannia). The hydrogenation proceeded for approximately 3 hours, or until the hydrogenation was complete. The hydrogenation mixture was flushed with nitrogen and filtered (Whatman 44 paper) to remove as much catalyst as possible. Remaining catalyst was removed by filtration of the solution a second time through a Millex HV filter (Waters Scientific, Milford, Massachusetts). The ethyl acetate was evaporated using a stream of nitrogen and the hydrogenated methyl esters were weighed.

2.3.3 Concentration of cyclic monomers by urea fractionation

The method of urea adduction (Firestone et al., 1961) followed by low temperature filtration was used to remove the bulk of the saturated linear fatty acids and hence to concentrate the cyclic fatty acids. A

mixture of hydrogenated methyl esters (1 part), urea (3 parts), and methanol (70 parts) was warmed on a hot plate to achieve complete dissolution of the solid material; the mixture was held at -20°C for at least 18 hours to enhance complexation. The mixture was then filtered at -47°C using a sintered glass funnel (Kimax 60 ml - 40M) which was modified so that a glass jacket surrounded the funnel. A mixture of dry ice and acetone was placed in the jacket to maintain a temperature of -47°C throughout the filtration. The methanol was evaporated and diethyl ether (4 x 5 ml) was used to extract the hydrogenated methyl esters. The diethyl ether was evaporated and the methyl esters were redissolved in hexane (1 ml) which contained a known quantity of internal standard (methyl undecanoate; NuChek Prep., Elysian, Minnesota).

2.3.4 Gas Chromatography - Mass Spectrometry (GC-MS):

The concentrated samples which were obtained by urea fractionation were analyzed using a Hewlett Packard GC-MS system; the system consisted of a 5890A Gas Chromatograph, a 5970 Mass Selective Detector, a 7946 Control System and a methyl-silicone cross-linked capillary column (0.2 mm ID x 12 m; 0.33 μ film thickness) which was directly coupled with the source of the mass spectrometer. An aliquot (1 μl) of the hexane solution was injected (splitless mode) at 270°C. The GC oven temperature was held at 60°C for 1 min and then it was programmed in 3 stages as follows: first, from 60°C to 160°C at a rate of 10°C/min, then from 160°C to 220°C at 5°C/min, and finally from 220°C to 290°C at 15°C/min. The mass spectrometer parameters were fixed automatically. All spectra were obtained at 70 eV in the electron impact ionization mode.

2.4 Fatty Acid Analysis

The preparation of methyl esters and the determination of fatty acid composition by capillary-column gas liquid chromatography was described in Chapter III.

3. RESULTS AND DISCUSSION

The total quantities of C18 cyclic fatty acid methyl esters in the various butterfat and vegetable oil samples are reported in Table 24. In all instances, the values reflect the sum of several C18 cyclic monomers with either propyl or butyl substituents. After both 8 and 16 h of heat treatment, all of the vegetable oil samples contained higher amounts of cyclic monomers compared to the butterfat samples. This would be expected because of the relative abundance of polyunsaturated fatty acids in the vegetable oils compared to the amounts present in the butterfat samples (Table 25). To the authors' knowledge, there are no reports in the literature of the formation of cyclic monomers from pure systems of saturated or monounsaturated fatty acids. Among the vegetable oils, the thermally oxidized corn oil had the lowest amount of cyclic monomers; there was very little difference between the amounts of cyclic monomers in the sunflowerseed, soybean, and canola oils (Table 24). Whole butterfat and the L-19 fraction from butterfat contained similar amounts of cyclic monomers (Table 24); the S-29 fraction had slightly lower values, especially after 16 h of heat treatment. Overall, the values reported for the vegetable oils are within the range reported by other workers (Gente and Guillaumin, 1977; Meltzer et al., 1981; Frankel et al., 1984);

Table 24. Cyclic Fatty Acid Monomers in Thermally Oxidized Butterfat, Butterfat Fractions and Certain Vegetable Oils.

Oil	Heating period (h)	Total C18 cyclic fatty acid methyl esters (%)
Whole butterfat	8	0.039 (± 0.001)[a]
	16	0.061 (± 0.003)
S-29 Fraction of butterfat[b]	8	0.035
	16	0.048 (±0.005)
L-19 Fraction of butterfat	8	0.038 (± 0.005)
	16	0.061 (± 0.003)
Sunflowerseed oil	8	0.13 (± 0.014)
	16	0.18 (± 0.052)
Soybean oil	8	0.13 (± 0.007)
	16	0.21
Canola oil	8	0.14 (± 0.003)
	16	0.21 (± 0.001)
Corn oil	8	0.060 (± 0.007)
	16	0.13 (± 0.001)

[a] The deviation is the average deviation from the mean of one sample, analyzed in duplicate.

[b] Fraction designation indicates physical state (solid, liquid) and fractionation temperature (°C).

Table 25. Fatty Acid Composition of the Unheated Butterfat, Butterfat Fractions and Vegetable Oils Which Were Used in the Study.

Fatty acid	Methyl esters (Wt %)						
	Whole butterfat[a]	S-29 fraction[b]	L-19 fraction[b]	Sunflowerseed oil[a]	Soybean oil[a]	Canola oil[a]	Corn oil[a]
Saturated	66.73	72.28	64.69	10.74	15.07	7.43	12.99
Monounsaturated	27.36	22.36	29.06	18.00	22.66	58.16	26.83
C18:1	23.86	19.44	25.21	18.00	22.57	55.69	26.83
Other	3.50	2.92	3.85	-	0.09	2.47	-
Polunsaturated	2.36	1.92	2.70	70.90	61.86	34.17	60.17
C18:2	1.57	1.33	1.82	70.69	53.17	24.44	59.47
C18:3	0.79	0.59	0.88	0.21	8.69	9.73	0.70
Other	3.55	3.44	3.55	0.35	0.42	0.24	-

[a] Each value represents the mean of one sample, analyzed in duplicate.

[b] Each value represents the mean of 3 replicate samples, analyzed in duplicate.

direct comparisons cannot be made, however, due to differences in heat treatments and analytical methods that have been used by the various investigators.

In a previous study (Gente and Guillaumin, 1977) of cyclic monomer formation in heated vegetable oils, estimations were made by direct gas chromatographic (GC) analysis of the hydrogenated fatty acid methyl esters. The components which eluted after methyl stearate but prior to methyl eicosanoate were considered to be the cyclic monomers. Meltzer et al. (1981) found that at low levels of cyclic monomers (less than 0.5%) it was necessary to concentrate the samples by low temperature crystallization of the hydrogenated fatty acids before accurate estimations could be made. Again, the final estimation of cyclic monomers by these authors, was performed by GC analysis (flame ionization detector) taking into account the peaks which eluted between C18:0 and C20:0 on the basis of retention time; it was confirmed by GC-MS analysis that the peaks which eluted between C18:0 and C20:0 contained many isomers (some of which were not fully resolved) of C18 cyclic monomers. In the present study, it was also necessary to concentrate the samples for detection and estimation of cyclic monomers; a urea fractionation technique was used. The estimation of cyclic monomers, however, was performed directly from the total ion scans (ion range 50 to 300 a.m.u.) obtained by the mass selective detector. Only the peaks which were identified to be cyclic monomers by selective ion monitoring (SIM) were included in the calculations. This was necessary in the analysis of butterfat samples because of the complexity of the composition of fatty acids in butterfat. Unheated butterfat is known to contain small or trace quantities of

several branched chain fatty acids and at least one cyclic acid (Patton and Jensen, 1975). These are concentrated along with the cyclic monomers by urea fractionation and can elute from the GC column in similar positions as the cyclic monomers.

Figure 15 shows the GC-MS identification of cyclic monomers in a thermally oxidized S-29 fraction of butterfat. Methyl undecanoate was selected as an internal standard since it eluted in a region of the chromatogram which was relatively free from interfering peaks. Identifications of cyclic acid peaks were made by computer-assisted plots of selected ions which were reported by Meltzer et al. (1981) as being characteristic of cyclic acids with propyl or butyl substituents (Fig. 16). Cyclic acids with a propyl branch were thus identified by plotting characteristic ions at m/z 296 (M+), 253 (296 - C_3H_7), 221 (296 - C_3H_7, CH_3OH), and 203 (296 - C_3H_7, CH_3OH, H_2O). Cyclic acids with a butyl branch were identified by a characteristic ion at m/z 189 (296 - C_4H_9, CH_3OH, H_2O) rather than at 203. If these assumptions are correct, there are two or three cyclic acids with a butyl chain present (Fig. 15(b), Fig. 17). Whether the ring contains less than 6 carbons or if there is branching present on the side chain could not be determined from the mass spectra which were very similar (data not shown). These compounds would need to be isolated for further characterization.

Some major differences occurred between the analysis of cyclic monomers in butterfat and in vegetable oils. In two of the samples (sunflowerseed oil and corn oil), one of the cyclic acids with a butyl side chain (peak D) eluted from the GC column along with the propyl branched

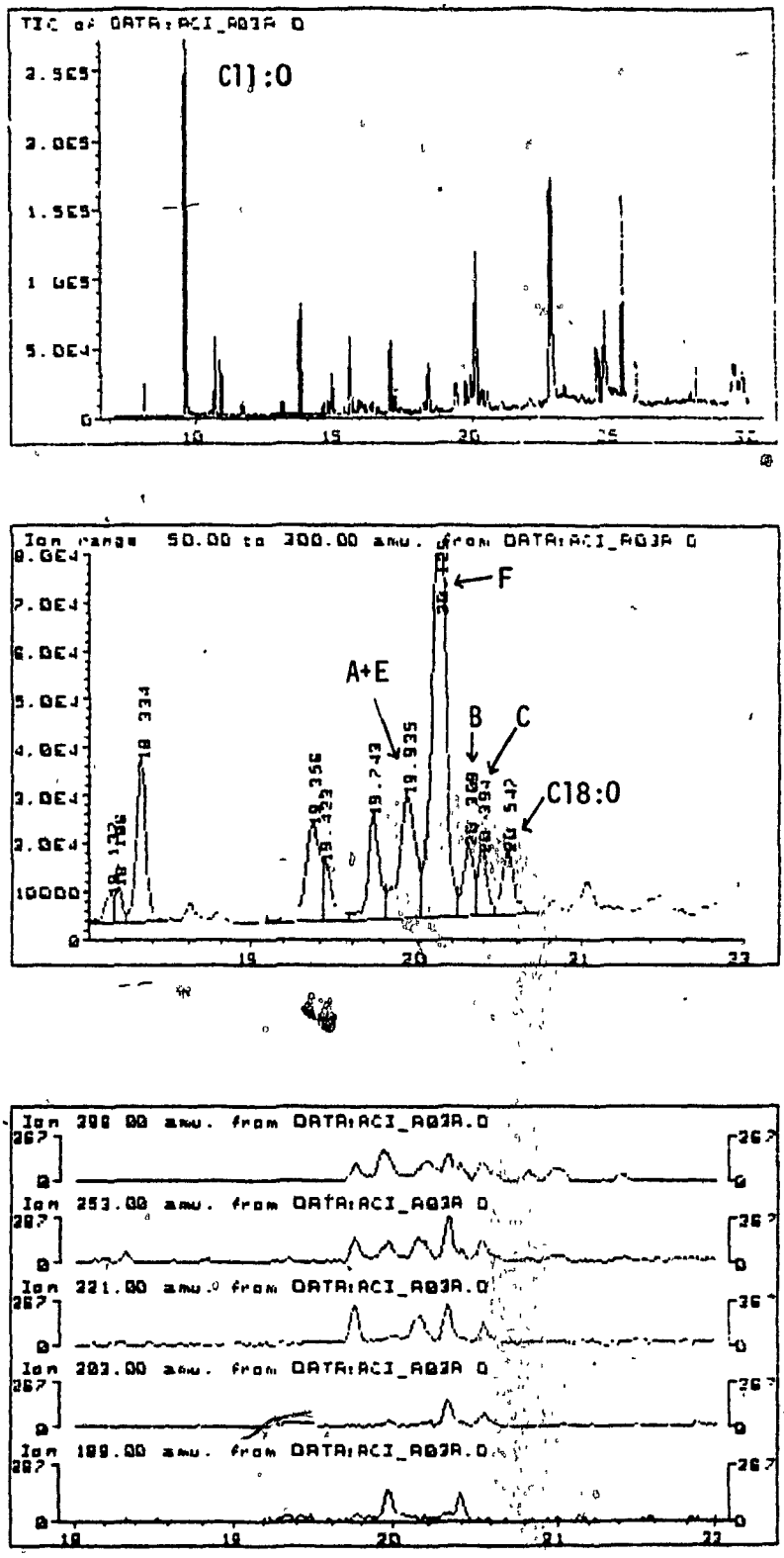
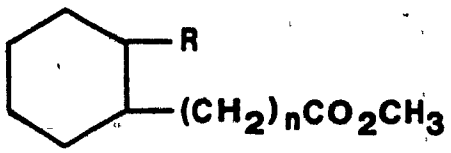


Fig. 15. GC-MS identification of cyclic monomers in a thermally oxidized (185°C; 16 h) S-29 butterfat fraction; (a) Total ion chromatogram (TIC) of the non-urea-adductable fraction of hydrogenated methyl esters, (b) expanded region of the TIC between 8 and 22 min, (c) computer-assisted selected ion monitoring (SIM) analysis of the expanded region.

M+ 296



Propyl	Butyl
$R = C_3H_7$	$R = C_4H_9$
n = 8	n = 7

Fig. 16. General structure of a C18 cyclic acid with propyl or butyl substituents.

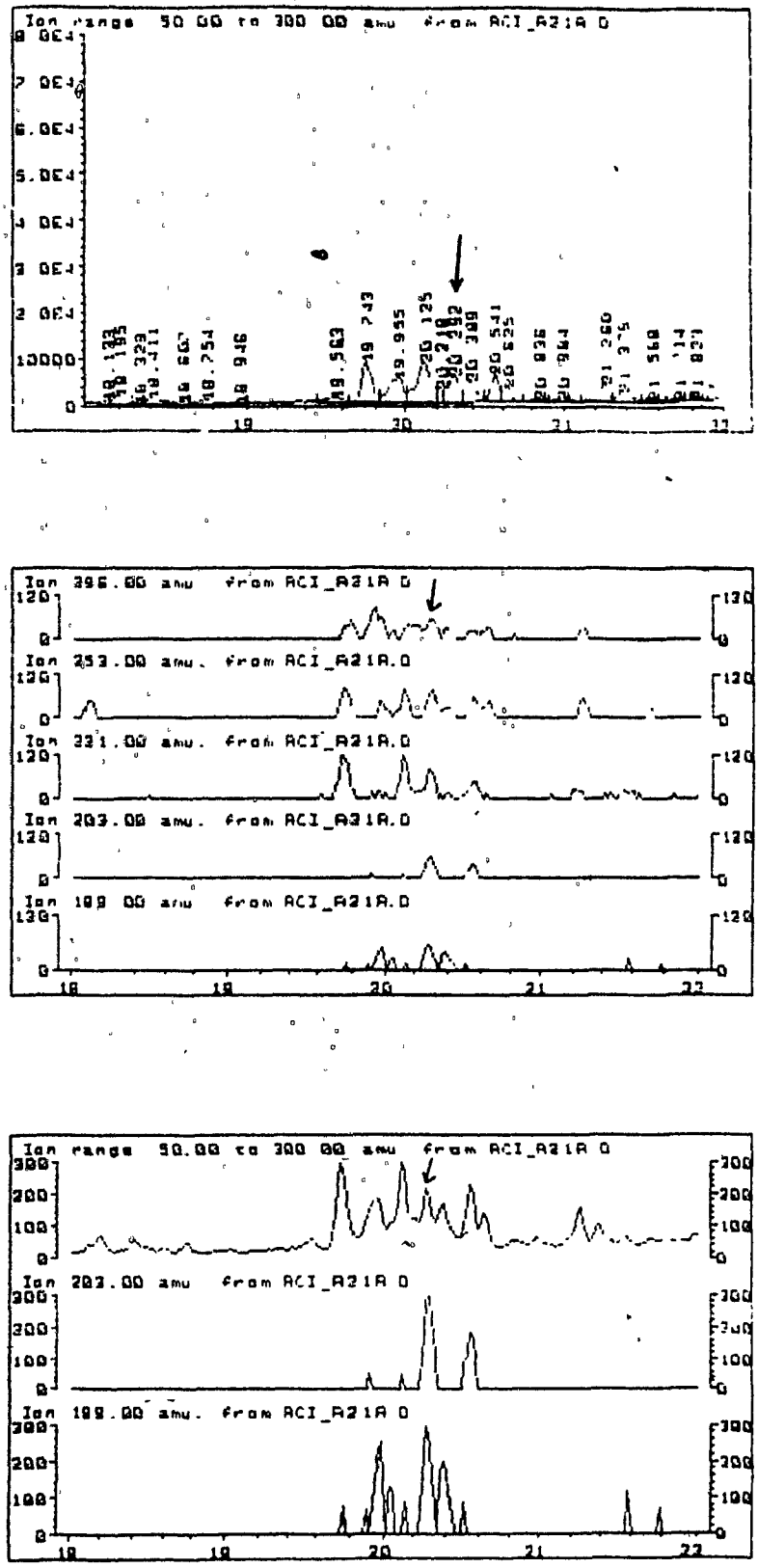


Fig. 17. GC-MS identification of cyclic monomers in thermally oxidized (185^{o}C; 8 h) corn oil. SIM analysis which shows that a butyl and a propyl branched C18 cyclic fatty acid eluted together.

cyclic acid (peak B) as was indicated by the SIM analysis (Figure 17). With the butterfat samples, the first cyclic acid with a butyl chain (peak A) was present in trace amounts. A compound (Fig.15(b), peak E) which interfered with this cyclic acid in the butterfat samples was identified as 11-cyclohexylundecanoic acid (M+ 282); the mass spectrum was identical to the one given by Schogt and Haverkamp Begemann (1965) for this cyclic acid which was isolated from butter (Fig.18). There was also a methyl ester of a branched chain fatty acid with 20 carbon atoms (Fig.19) which eluted in the same region as did the cyclic acids in the butterfat samples (Fig.15(b), peak F); chain branching was easily determined by the characteristic ions at m/z 101, 171, 241 and 311 which corresponded to the sites of cleavage at the points of branching. If there was no methyl group at C3, the ion at m/z 87 would appear, rather than the ion at m/z 101. This branched chain fatty acid (3,7,11,15 - tetramethylhexadecanoic acid) was isolated previously from butterfat by Sonneveld et al. (1962).

The whole butterfat (unheated) was analyzed (data not shown) to determine whether or not C18 cyclic monomers existed in the fat prior to thermal oxidative treatment. The GC-MS analysis confirmed the presence of both 11-cyclohexylundecanoic acid and 3, 7, 11,15 - tetramethylhexadecanoic acid in the unheated butterfat sample. No C18 cyclic fatty acids were detected in the unheated butterfat.

In summary, the present study has shown that C18 cyclic fatty acids do not occur in unheated butterfat but they are formed in butterfat and fractions of butterfat as a result of heating (185°C, 8 or 16 h) in the presence of air. The levels at which these compounds are formed,

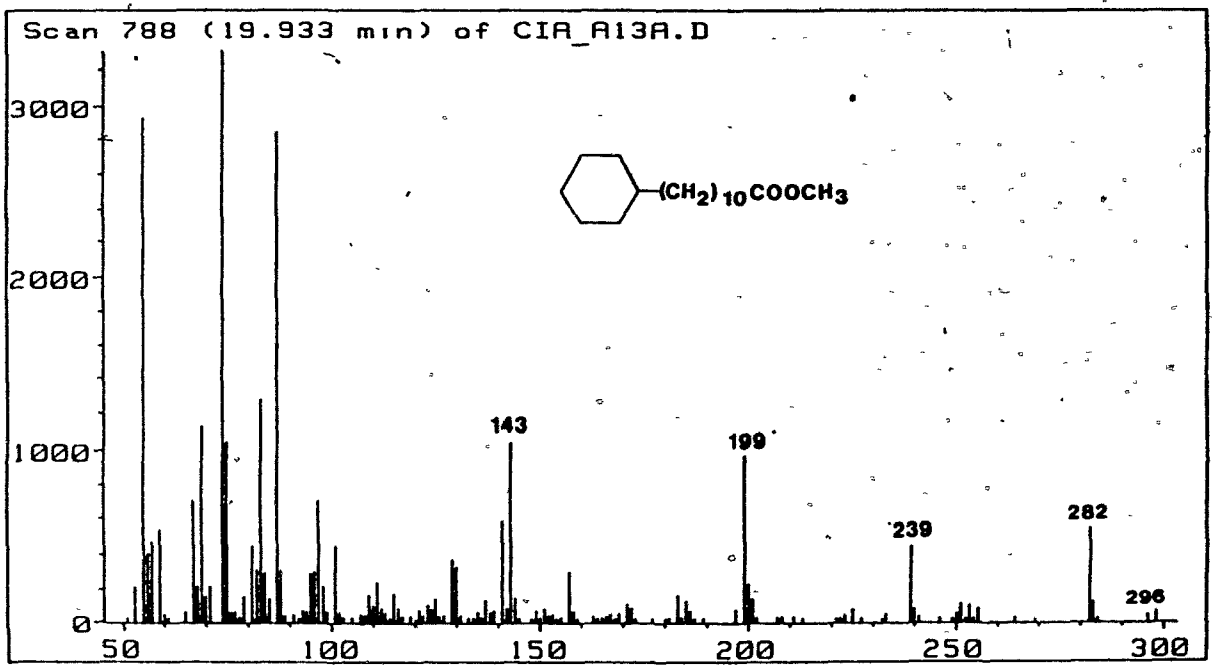


Fig. 18. Mass spectrum of the peak which corresponds to 11-cyclohexylundecanoic acid methyl ester (M+ 282) (contaminated by acid M+ 296).

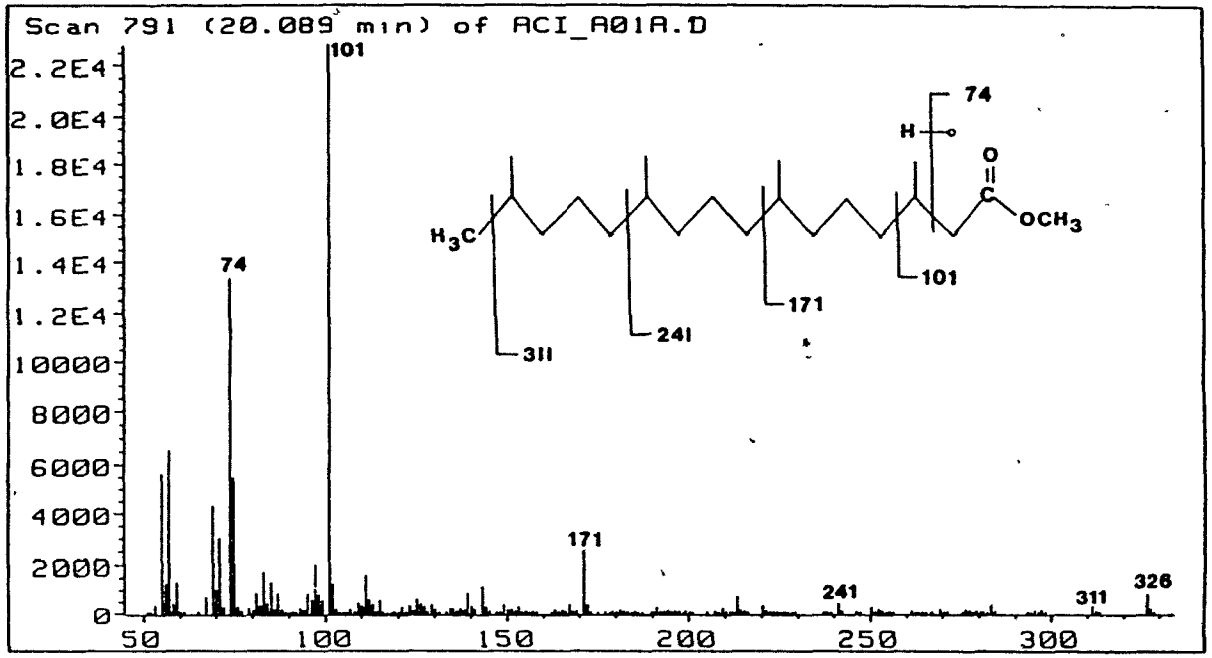


Fig. 19. Mass spectrum of the peak which corresponds to 3,7,11,15-tetramethylhexadecanoic acid methyl ester.

however, are considerably lower than the amounts which were detected in vegetable oils which were treated under identical conditions (Table 24). It is reasonable to expect that only the polyunsaturated fatty acids give rise to cyclic monomers; thus, the levels at which these compounds can form in butterfat as a result of heating is limited by a low level of polyunsaturated fatty acids in the fat (Table 25). The cyclic monomer fractions in all of the samples were identified by GC-MS to contain primarily disubstituted C18, cyclic acids with butyl or propyl substituents. It has not been established whether these cyclic acids occurred as free fatty acids or as constituents of glycerides. The method of analysis also did not permit the number and location of double bonds in the structures to be established. The data obtained from the analysis of the unheated and heated butterfat samples clearly demonstrate the importance of mass spectrometry in the identification of cyclic monomers in complex fats. Unless the components of the sample are adequately resolved (by a capillary column) and accurately identified by GC-MS, the amounts of cyclic monomers in heated butterfat may be overestimated because of the naturally occurring cyclic and branched chain fatty acids which can elute in the same region of the chromatogram as do the C18 cyclic acids.

CHAPTER VII

GENERAL DISCUSSION AND DIRECTIONS FOR FUTURE RESEARCH

In the investigations reported in this thesis, the thermal oxidative behaviour of butterfat was compared to that of certain vegetable oils, i.e., soybean, sunflowerseed, canola and corn oils. Butterfat was studied because of its importance as a food fat and the lack of information in the literature regarding its thermal oxidative behaviour. In addition, a procedure was developed for the fractionation of butterfat and the thermal oxidative behaviours of the resultant fractions (solid and liquid) were studied. These fractions might find use in various food applications and thus, information about their thermal oxidative stability compared to that of whole butterfat, is important.

The technique which was devised for the fractionation of butterfat (Chapter III) involves two distinct processing steps: (i) the partial crystallization of molten butterfat under controlled conditions of time, cooling rate and agitation to yield a mixture of large crystals suspended in liquid oil, and (ii) separation of the solid and liquid fractions by vacuum filtration using a specialized apparatus. Experiments are reported in Chapter III which describe the fractionation of winter butterfat at 29, 26, 23 and 19°C and summer butterfat at 29 and 19°C. The technique was reproducible, yielded fractions which differed markedly in their chemical and physical characteristics, and the processing steps did not cause oxidative deterioration in the resultant fractions. Subsequent

thermal oxidation experiments were performed on the fractions obtained at 29 and 19°C. The S-29 and L-19 fractions possessed the widest possible differences in chemical and physical characteristics which are attainable when butterfat is fractionated by crystallization from molten fat and in general, there were no significant differences in fatty acid compositions between fractions obtained at 29 and 26°C and between those obtained at 23 and 19°C (winter butterfat experiments).

The results of the thermal oxidation experiments (Chapter IV, V and VI) indicated that butterfat and the fractions of butterfat are much more stable to thermal oxidation than are canola, sunflowerseed and soybean oils. This was evidenced by substantially higher contents of inter- and intramolecular polymers, total polar components, and C18 cyclic monomers in the vegetable oils than in any of the butterfat samples after both 8 and 16 h of heating at 185°C. The corn oil also exhibited a high degree of stability after 8 h of heating when the levels of inter- and intramolecular polymers, and total polar components are considered (Chapters IV and V). The corn oil, however, contained higher levels of C18 cyclic fatty acids (after both 8 and 16 h) than did any of the butterfat samples (Table 24). As stated earlier, the results of the corn oil which was heated for 16 h are somewhat uncertain due to the presence of a very viscous and dark coloured material which could not be removed from the inner walls of the oxidation flask. This material represented 2 - 3% of the original weight of the sample and was believed to contain highly polymerized oil.

There is no doubt that a large part of the differences observed in the thermal oxidative behaviours of the butterfat samples in comparison to the soybean, sunflowerseed and canola oils stem from basic differences in the fatty acid compositions of the unheated fats and oils (Tables 8-11, 16 and 25). From studies of pure systems of triglycerides or fatty acid esters, it is known that the oxidative stability of polyunsaturated fatty acids is considerably lower than that of monounsaturated fatty acids which are in turn less stable than saturated fatty acids. In fats and oils, however, where a variety of fatty acids are arranged as triglycerides and other components are present (e.g., unsaponifiable components), other factors are also important in determining the thermal oxidative stability as was indicated from the results with corn oil (8-h heat treatment) and the L-19 butterfat fractions.

The high degree of stability of corn oil during 8-h of thermal oxidation could be due to the action of naturally occurring antioxidants during the early stages of heating. Corn oil contains a relatively high tocopherol content (in mg/kg of oil: α, 191; β, trace; γ, 942; δ, 42) and very small amounts of another antioxidant component, ferulic acid (Sonntag, 1979). Further studies are needed to determine the composition of vitamins in the fats and oils which were used in the present investigations and their relationship to the thermal oxidative behaviour of the fats and oils. Yoshida and Alexander (1982) have shown that corn oil is degraded to the same extent as sunflowerseed oil and more than soybean oil by thermal oxidation at 180°C for 50, 70, and 100 h (based on total polar components). The heating times in this study were much longer than those used in the present experiments and could account for

the differences in the results of the two studies. It is possible that if the fats and oils in the present study, were heated beyond 16 h, the extent of degradation of the corn oil would have reached that of the other vegetable oils, while the degradation in the butterfats would start to level off. There is some indication of this from the results (Tables 15 and 24) which showed larger differences between the 8- and 16-h corn oil data than between the 8- and 16-h butterfat data.

With the winter butterfat samples, after 8 h of thermal oxidation, both the solid and liquid butterfat fractions exhibited more stability toward intermolecular polymerization than did the whole butterfat (Table 17). After 16 h of heating, however, the extent of intermolecular polymerization increased with increasing degree of unsaturation of the fat. With the summer butterfat samples, the degree of intermolecular polymerization was related to the degree of unsaturation of the fat, after both 8 and 16 h of heat treatment. When the total polar components in the heated fats were assessed, however, the liquid fractions obtained from the summer butterfat showed some stability to thermal oxidation (after both 8 and 16 h) as compared to the whole butterfat (Table 21). The whole butterfat contained a higher proportion of oxygenated triglycerides and hydrolysis products in its degradation products while the liquid fractions contained a higher proportion of polymeric triglycerides in their degradation products. Thus, consideration of only one indicator of fat deterioration such as the amounts of polymeric triglycerides in the heated fat may not give the complete picture. The amounts of C18 cyclic fatty acid monomers were the same in the whole summer butterfat and the liquid butterfat fractions.

The greater stability of the solid butterfat fractions to thermal oxidation compared to the whole butterfat was most likely the result of the lower degree of unsaturation of the solid fractions and their greater content of trisaturated glycerides (Chapter III). The stability of the liquid fractions, however, was most likely the result of the presence of an "antioxygenic" factor or naturally occurring antioxidant(s) which become(s) concentrated in the liquid fractions by the fractionation process. It is not possible to state from the results of the present experiments what the nature of this antioxidant or "antioxygenic" factor might be. Further research is needed to characterize the component(s) responsible for the high degree of stability of the liquid fractions and to elucidate its mode of action.

Vitamin A and β - carotene are known to be concentrated in the liquid fractions during fractionation of butterfat (Norris et al., 1971). There are contradictory reports in the literature, however, on whether these compounds are antioxidative, prooxidative, or without effect on butterfat oxidation (Dugan, 1980; Eriksson, 1982).

Several other components of milk or milkfat are believed to have antioxidant properties; these include phospholipids (Pruthi et al., 1970 and 1971; Bector and Narayanan, 1972), sulfhydryl groups (El-Rafey et al., 1944; Taylor and Richardson, 1980), casein (Taylor and Richardson, 1980) and browning reaction (carbonyl-amine reaction) products (Dugan, 1980; Eriksson, 1982). The effects of these components are, however, more important when the fat exists in milk or when the processing conditions favour their transfer to anhydrous milkfat or butterfat. In the present study, the process which was used to isolate anhydrous butterfat from

butter included minimal heat treatment and did not favour the presence of residual non-fat solids in the butterfat. Also, the phospholipid content is greatly reduced in butterfat during the buttermaking process (Lampert, 1975).

One class of compounds which occur naturally in butterfat and would be of interest to study with respect to possible antioxidant activity, are the lactones. Lactones are important contributors to milk flavour. They are formed in butterfat from the γ- and δ-hydroxyacids and their formation is greatly enhanced at elevated temperature (Urbach, 1979). According to Walker (1974), the precursors of lactones and methylketones are mainly transferred to the low-melting fractions during butterfat fractionation. Dziedzic and Hudson (1984) demonstrated that phenolic lactones (e.g., aesculetin) when added to edible oils, possess some antioxidant character. Essential molecular features of the compounds which were studied by Dziedzic and Hudson (1984), which contributed to a high level of antioxidant activity included: (i) at least two or more neighbouring phenolic hydroxyl groups, and (ii) a carbonyl group, in the form of an aromatic acid, ester or lactone, or a chalcone, flavanone or flavone. The lactones which form in butterfat do not meet the first criteria, however, they may become hydroxylated during thermal oxidative reactions.

Bhalerao et al. (1959) assessed the nutritional value of thermally oxidized butterfat fractions obtained by crystallization from acetone or alcohol and, of certain vegetable oils. The authors fed thermally oxidized (200°C for 24 h) butterfat, acetone or alcohol-soluble and insoluble fractions (0 and 20°C) of butterfat, corn oil or

hydrogenated soybean oil to rats for a four week period. The animals which were fed the thermally oxidized butterfat or the acetone-insoluble fraction of butterfat did not exhibit any differences in growth when compared with animals which were fed the corresponding fresh fats. The alcohol-soluble or alcohol-insoluble fractions of butterfat produced slight growth depression while marked growth depressions were observed in rats consuming the acetone-soluble fraction of butterfat or the vegetable oils. When the vegetable oils were mixed with 30% of the acetone-insoluble fraction before thermal oxidation, there was no difference in the growth of rats compared to those consuming fresh fats. The authors concluded that butterfat contains relatively stable triglycerides (such as trisaturated glycerides) which are acetone-insoluble and which are able to counteract the effects of toxic products or prevent the formation of toxic products during heating. From the knowledge that has been accumulated about the toxic components in heated fats and oils since the Bhalerao et al. (1959) study, it is reasonable to assume that the toxic effects of the heated oils were related to the levels of cyclic monomers in the oils. The present study has shown that the content of cyclic monomers in the thermally oxidized S-29 fractions is slightly lower than that of the L-19 fractions or whole butterfat which were treated under similar conditions (Table 24). All of the butterfat samples had considerably lower contents of cyclic monomers compared to the vegetable oils (Table 24). The differences between the solid and liquid fractions in the present study are not as pronounced as if fractions from acetone crystallization had been studied since the latter procedure yields a clearer separation between solid and liquid fractions. It is possible

that in the Bhalerao et al. (1959) study, the absence of toxicity symptoms which were observed when the acetone-insoluble fraction was blended with the vegetable oils prior to thermal oxidation, was purely due to a "dilution-effect" whereby the level of polyunsaturated fatty acids in the blended oil which were available for cyclic monomer formation, were reduced. Further research is needed to assess the nutritional value of the thermally oxidized fats and oils used in the present study.

The results from the present experiments, although they provide valuable comparisons between the effects of thermal oxidation on the constitution of butterfats and vegetable oils, cannot be extrapolated to the frying of foods since many other factors come into play when food is either pan fried or deep fried. Further studies are needed to assess the thermal oxidative behaviour of butterfat and butterfat fractions in actual frying experiments.

CLAIMS TO ORIGINALITY

The studies contained in this thesis make several contributions to original knowledge.

From the point of view of methodology, a unique method for the fractionation of butterfat by crystallization from molten fat has been devised. The technique is practical, reproducible, and yields fractions which differ markedly in their physical and chemical characteristics. In addition, the fractions are representative of those which could be obtained on a commercial scale. Thus the results of analyses (fatty acid and triglyceride analyses, thermal examinations by differential scanning calorimetry, melting point, iodine value and peroxide value) of the fractions obtained by this technique and the study of their thermal oxidative stability are meaningful and contribute to original knowledge.

Advantageous modifications have been introduced to the methodologies used for the evaluation of the thermal oxidative behaviour of vegetable oils and in the application of these methodologies to the study of thermally oxidized butterfat and butterfat fractions. Improved methodologies are described for the analysis of polymeric material and of C18 cyclic monomers in thermally oxidized fats and oils.

This is the first comparative study of the effects of thermal oxidation on the constitution of butterfat, butterfat fractions and certain vegetable oils (soybean, sunflowerseed, canola and corn oils). To the authors knowledge, there are no previous reports in the literature on the thermal oxidative behaviour of butterfat fractions. Furthermore, the studies which have dealt with the thermal oxidative behaviour of whole butterfat have concentrated primarily on the volatile decomposition

products. The present investigations deal with the nonvolatile degradation products which are formed during the thermal oxidation of butterfat.

This is the first demonstration, to the authors' knowledge, that the greater stability of butterfat to thermal oxidation compared to that of vegetable oils is not only due to the greater degree of saturation of butterfat but also to another factor(s) or component(s) as would be indicated from the results with the butterfat fractions in comparison to whole butterfat. This factor or component is concentrated in the liquid fractions during fractionation and thus contributes to a comparatively high degree of stability of the liquid fractions despite their increased unsaturation.

This is the first demonstration, to the authors' knowledge, that differences exist in the major routes of decomposition of solid and liquid butterfat fractions during thermal oxidation. Polymerization is a predominant decomposition pathway in liquid butterfat fractions while oxidative and perhaps hydrolytic reactions are more important in solid butterfat fractions.

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